

1 May 1980

Does science need a tax-break?

SHOULD President Carter introduce generous new tax incentives — in particular increased deductions for money spent on research and development — to help reverse the productivity decline widely claimed as a major source of the nation's economic problems? American business is convinced that he should: the Committee for Economic Development, for example, synthesising the views of many large corporations, said earlier this year that 'top priority' should be given to appropriate tax changes as a means of stimulating technological innovation.

The administration itself is less certain. While accepting the argument that tax relief for R and D could help increase the rate of innovation (and even this is yet to be supported by hard data) administration officials also point to the cost of such measures in terms of reduced federal revenues. Revisions to the tax law have long been promised, and many expected them to be included in the package of measures to spur innovation which the President presented to Congress last autumn. But such moves have since been overshadowed by attempts to balance the budget.

At present, although private companies can deduct R and D expenditures as running expenses, no special incentives exist for investment in R and D equipment. This is in contrast to France, for example, where accelerated depreciation is allowed on all investments in research. Or Germany, where a 7.5 per cent tax-free cash subsidy is granted on money spent on R and D facilities.

Current demands to reduce the tax burden represent the intersection of two trends. The first is a general political thrust to reduce taxes on both individuals and corporations, represented most vividly by a proposal before Congress for an immediate 30 per cent cut in personal income tax. The other is a more detailed argument that, in order to encourage more private R and D, it should be made more attractive than other possible investments — with the tax system being used to this end.

In general the two trends reinforce each other. But there are ways in which they could conflict. For example, political enthusiasm for tax relief to major corporations has produced broad-based support for a bill introduced by representatives James R Jones and Barber B Conable which would shorten the time over which companies could write off capital investments. Rather than using a 'useful life' formula which differs from industry to industry, the bill proposes fixed depreciation times of ten years for buildings, five for machinery and equipment, and three for light vehicles (hence referred to as the 10-5-3 proposal).

Supporters of the bill, which has more than 300 co-sponsors in the House of Representatives and would include R and D equipment, argue that it would free capital for more lucrative investment, and is thus precisely the type of measure needed to raise the general level of economic activity. Critics, however, have suggested that the benefits of such a bill would be skewed in favour of capital-intensive industries, and not necessarily high

technology firms with large R and D costs, where the 'write-off' time may already be short. It is also argued that the Jones-Conable bill would cost \$5 billion in the first year, and \$25 billion after five years — and that the resultant inflationary pressures could outweigh any benefits.

In the light of such objections, a separate proposal has been made by representative Charles Vanik of Ohio. He has proposed a Basic Research Revitalisation Act which would offer tax incentives to companies supporting university research. In particular, companies would be given a 25 per cent tax credit on money deposited in a special research reserve (up to five per cent of the company's business income). And any money taken from this reserve to fund basic or 'exploratory' research in universities could be claimed as a tax deduction, providing it was spent within four years.

Research universities are understandably enthusiastic about the Vanik bill, which has been co-sponsored by over half the members of the House Committee of Science and Technology. As funds for federal support for basic research grow tighter, universities are increasingly looking to industry as a source of financing. A typical example is the announcement expected this week from the Massachusetts Institute of Technology that it is to receive a large grant from the Exxon Company to support fundamental combustion research. Exxon officials have already spoken in support of tax relief for such moves, claiming it would encourage other companies to follow their example.

But the question hanging over the Vanik proposals is whether they would make any significant impact on the problem they are intended to address, namely the nation's declining productivity and the consequent slowdown in economic growth. Most analysts agree that it is not support for R and D itself which is missing, but adequate mechanisms for translating knowledge into useful and acceptable products. A report published last week by the National Science Foundation, for example, shows industrial R and D to be in a surprisingly healthy state, experiencing a five per cent real growth between 1976 and 1977, with a 22 per cent increase in the number of qualified scientists and engineers employed between 1972 and 1977.

Demands for tax incentives to stimulate research, particularly made at a time when the overall level of R and D effort would appear adequate, reflect a desire to shift the control of research funds from the public to the private sector. Such a move has obvious attractions to those who would benefit most directly; but whether it is the best solution is a political, not a technical, judgement. The success of Japanese industry in fields from motorcycles to digital watches has been achieved in active partnership with government. In Britain the Spinks report on biotechnology endorses a similar marriage. In the US, the key to rekindling innovation should be better government — not less. □



United States

An excess of doctors...

David Dickson reports from Washington on the levels of manpower in medicine, and womanpower in science

AFTER fifteen years' rapid expansion, US medical schools are beginning to produce more doctors than the country needs. Earlier this month the Department of Health, Education and Welfare (DHEW) predicted that the surplus could be between 4,000 and 40,000 by 1990; and other estimates make it considerably higher.

Numbers alone, however, hide the fact that not all medical graduates go where they are required. In particular, the more lucrative fields of surgery continue to exert higher attraction than primary health care, especially on those who may leave medical school with outstanding loans of \$40,000 to \$50,000.

Ironically the current situation is largely the result of the success of the government's previous efforts to fit medical education to social needs. These were initiated in the late 1960s, when a national shortage of doctors led the government to offer generous grants to medical schools willing to increase their enrollments.

With the encouragement of 'capitation' grants (based on the number of students) and funds for the creation of new medical schools, the number of places for medical students has doubled, from about 8,000 to 16,000. And although the rate of expansion has slackened off, there is no indication that it is likely to fall, particularly given the continued excess of applicants over available places.

The consequence of a growing domestic supply plus the influx of foreign doctors during the period of shortage is that, according to figures prepared by DHEW's Bureau of Health Manpower, there will be 600,000 doctors in the US by 1990 — but the need will only be for between 553,000 and 596,000.

Congress' Office of Technology Assessment (OTA) predicts an even wider gap. In a report on the bureau's statistical methodology published last week, it agrees with the supply figure but points out that the projected need is based on a linear extrapolation of an observed increase in the per capita demand for physicians over the period 1968 to 1976.

The OTA report also points out, however, that since 1971 the per capita demand has been falling. And that if this trend is combined with expected demographic changes, the need for doctors by 1990 could be as low as 415,000.

No-one is particularly worried about the surplus itself. Despite the growing signs of a shift away from surgery into general practice, there are still likely to remain — as

both the DHEW and the OTA report emphasise — fields in need of more doctors, for example, for work among immigrant and minority populations.

The dilemma is over the appropriate response of both the federal government and the medical schools. The Carter Administration is using the over-supply figures to support its proposal that all direct federal aid to medical schools should now be dropped, confining assistance to student grants and loans.

The schools, in contrast, feel that relying on tuition fees alone would place unacceptable pressures on students. And that some form of additional support, not tied to providing health services or to funding research which are both tightly regulated, would now be welcome.

So far the administration's attempt to eliminate capitation grants has met limited success. Although omitted from DHEW's budget request for 1980 last year, they were put back by Congress reacting to pressure from the medical schools. In January the President asked that the capitation grants be withdrawn from the 1980 budget, but again Congress disagreed. A further attempt to cut the money out was made in the President's revised budget request last month among the revisions for 1980. And the issue remains unresolved.

However congressional attitudes are changing, reflecting the schools' acceptance of the current illogic of measures to increase doctor supply. Last week a House subcommittee proposed that, in renewing the health education support legislation which runs out this year, capitation grants should be 'phased-down' by steps of 25% over the next three years. And although remaining agnostic over whether the nation faces an oversupply of doctors, the subcommittee agreed that no new medical schools should receive federal funding.

The main problem facing the schools is the implication of the growth in tuition fees, which can now go as high as \$8,000 a year. One approach has been to seek new forms of outside assistance; thirteen private medical schools announced last week that they are to divide \$1.3 million next year awarded by the Henry J. Kaiser Foundation to assist students with more forthcoming later to those able to raise matching grant from alumni or elsewhere.

Others are seeking new forms of federal support for teaching. "One way would be to develop a two-part system. All schools would get a certain base amount, and you could then pick and choose programmes which might qualify for additional funding" says Dr Richard Ross, Dean of the Medical Faculty and Johns Hopkins Medical School.

Without some such support, fears are growing that many of the trends which federal funding has encouraged — such as the growing recruitment of students from minority or low-income groups — could be reversed. And that current mismatches between the supply of physicians and health needs may only be exacerbated. □

... and a dearth of women

A political struggle appears to be looming on the Washington horizon over the steps that the US government should take to encourage the participation of women in science.

In passing the National Science Foundation's budget for 1981 last Thursday, the Senate Subcommittee on Health and Scientific Research included a provision for \$23 million to be allocated to a new 'women in science' programme within the foundation.

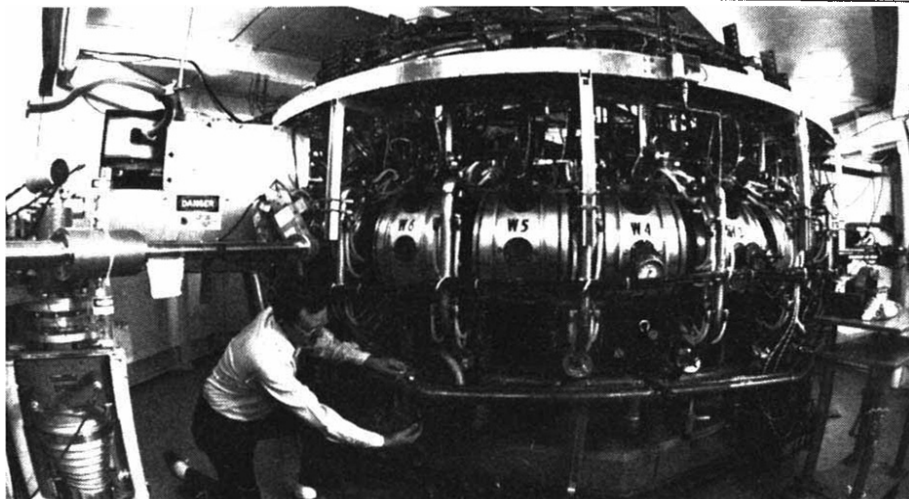
The elements of the programme are broadly similar to those included in a bill introduced last year by Senator Edward Kennedy. They include a new centre for women in science, a special committee within the foundation to examine the position of women scientists, the creation of appropriate university positions, and the appointment of a special assistant to the NSF director.

Subcommittee members say the need for such a programme is shown by the fact that the number of women in science remains

disproportionately low. But to make room for the programme while remaining under the revised ceiling for the NSF's budget proposed by President Carter earlier this month, the committee is recommending a *pro rata* 9% cut on all items listed in the original January budget request.

Although the impact on basic research programmes would be mitigated by the transfer of money that would have been spent on upgrading research facilities, this would cut back their increase from 11% to 7%. Funds for mathematical and physical sciences, for example, would fall from a proposed \$260 million to \$244 million.

The corresponding House Subcommittee, in voting on the NSF budget last week, kept closer to the administration's revised request for \$1,074 million. However, it added applied research and science education items, and cut back the proposed government/industry ocean margin drilling project from \$5 million to a \$500,000 study by the National Academy of Sciences. □



ELMO Bumpy Torus: dark horse of fusion

To most nuclear engineers, magnetic confinement fusion technology tends to be identified with the familiar doughnut shape of the Russian-designed Tokamak, which gained acceptance over US designs in the early 1960s, and of which 40 are now in experimental operation world wide.

But both Tokamak technology and the less familiar mirror confinement techniques still face many unanswered questions. And two years ago an *ad hoc* review committee appointed by the US Department of Energy recommended that, in the light of such uncertainties, increased support be given to alternative designs.

Two contenders emerged from a further review. One was the reversed field pinch, an adaptation of the Tokamak design; the other was a device that had been worked on at the Oak Ridge National Laboratory since the 1960s, known as the ELMO Bumpy Torus (EBT). A hybrid between the Tokamak and mirror designs, the EBT was subsequently selected for development to a point where it can be compared directly to the operation of existing Tokamaks.

Last Autumn, four industrial consortia — headed by Westinghouse, Grumman, McDonnell-Douglas and Ebasco — produced separate plans for a 'proof-of-principle' EBT. These were amalgamated into a single design. And earlier this month the four consortia were asked to bid competitively for what has become known as the EBT-P.

The Bumpy Torus is based on a discovery made by a team headed by Dr Ray Dandl at Oak Ridge in the 1960s. When magnetic 'mirrors' produced by current-carrying coils are used at either end of a cylindrical cavity to keep plasma particles from escaping, and the plasma is heated by radio waves at the electron cyclotron frequency, a ring of electrons is found to form around the plasma at the mid-point between the two end coils.

This ring is of sufficiently high energy to

keep the plasma away from the walls of the cylinder, necessary because of its high temperature. And the ring remains in place even when the end coils are inclined at an angle of 15 degrees to each other.

Putting 24 such cylindrical modules together end-to-end results in a torus. It is called 'bumpy' because the magnetic field is narrower between the mirrors than in the centre of each segment. No-one except Ray Dandl knows why it is called ELMO and he is not saying.

So far two experimental EBTs have been built at Oak Ridge (and one in Japan), and both have operated successfully according to theoretical predictions.

Operationally the EBT has two important advantages over the Tokamak, according to Dr John Sheffield, head of the Experimental Confinement Section at Oak Ridge. The first is that it operates in a steady state, rather than the pulsed mode of the Tokamak.

The second is that, without the complexity of the interlocking coils required by the Tokamak design — and the engineering difficulties caused by their magnetic interaction — construction problems could be considerably less.

Whether these advantages are sufficient to overcome the relative lack of knowledge about the EBT compared to the extensive amount already known about the behaviour of Tokamaks remains to be seen. At present EBT technology is about ten years behind; and with Congress pushing the DOE hard for the construction of an engineering test facility (ETF) for the Tokamak, it will be difficult to catch up.

Furthermore, promising results are being reported from tests with mirror confinement devices at the Lawrence Livermore Laboratory, three to five years behind the Tokamak but still ahead of the EBT. DOE officials are considering putting a request for \$100 million to construct a new mirror test facility in the 1982 budget. □

Senate approves patent changes

HOPES are high among US research universities that, following a six-year campaign, the US Congress will soon pass legislation boosting their rights to benefit directly from the results of research carried out with federal funds.

Last week the Senate passed a bill which would allow universities and small businesses, under certain defined conditions, to license any patents arising from such research. Similar measures are already under active consideration in the House of Representatives. And given the large majority that voted for the Senate measure, university lobbyists in Washington are confident that they will have a new law on the books by the end of the year.

Revision to the patent law has been a cornerstone of attempts both in Congress and by the Administration to tackle the recent drop in industrial productivity and an apparent decline in the rate of technological innovation. Both are claimed to contribute to the nation's economic problems. And both, it is argued, could be reversed by assisting the flow of research results into the market-place.

A variety of bills has been introduced into both legislative bodies over the past year. Some, such as a bill passed by a subcommittee of the House Science and Technology Committee three weeks ago, have recommended that patent rights be awarded to anyone carrying out federally-sponsored research.

The universities, however, fearful that too broad an approach might fail to win sufficient support, have put their efforts behind a more limited bill introduced by senators Birch Bayh and Robert Dole which gives patent rights to universities and small businesses, but excludes large businesses.

Six weeks ago, when the bill reached the floor of the Senate, it was threatened with a filibuster by Senator Russell Long, who claimed that liberalising the patent laws would encourage monopoly practices and discourage competition. The bill was subsequently withdrawn from debate.

When offered last Thursday, Senator Long spoke strongly against the bill. But he did not offer any amendments that might have killed the measure, and the final vote was 91 to 4 in favour, an indication of the depth of support.

Under the Senate Bill, a university will have the exclusive rights to a 17-year patent on federally sponsored research results, providing it illustrates that it has the institutional means to license the patent. The bill contains a provision that if the patent proves commercially successful, the government should be paid back for the costs of the research. **David Dickson**

United States

Asbestos: reductions urged

SCIENTISTS from the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health have urged further stringent reductions in the permitted exposure limits to asbestos fibres in the air.

Since 1972, the maximum permitted concentration in the work-place has been two million fibres per cubic metre in air. The scientists have now issued a report recommending that this exposure level be reduced to 100,000 fibres per cubic metre — the lowest level that can be accurately measured — and have urged a ban on all non-essential uses of asbestos.

At a news conference in Washington last week, Dr Eula Bingham, Assistant Secretary of Labour for Occupational Safety and Health, said that the agency intended to push shortly for reduced exposure limits — but refused to say whether it would endorse the study group's recommendation or a higher one.

Mind the spores!

THE US Defense Department published details last week of experiments carried out in the mid-1960s to test the New York subway system as a method for dispersing bacterial weapons.

According to the defence scientists who carried out the tests, commuters using the subway system paid little attention when they were sprayed with harmless strain of the bacterium *Bacillus subtilis* through gratings in the sidewalk, even though the aerosol clouds were clearly visible.

This led the research workers to the conclusion that the subway system, where the draughts caused by trains would disperse the biological agents over a wide area, could prove an effective way of inflicting severe casualties on civilian populations.

Particularly effective were light-bulbs containing the test bacilli which were dropped from moving trains within the subway system. According to the Defense Department report, released after a Freedom of Information request from the Church of Scientology, the bacteria "aerosolised and dispersed rapidly by the movement of trains, penetrating stations and trains in the area and persisting there for one hour or longer."

The report says that information about the effectiveness of subway dispersion had both defensive and offensive value.



"Forget the heroin business, Bugsy! I've something more profitable lined up!"

Interferon sales \$2 billion-plus?

ACCORDING to a report prepared by the US brokerage and investment firm Bache Halsey Stuart Shields, interferon sales could have a world wide market of \$2 billion a year within three to five years. The report, summarised in *European Chemical News*, specifies three major health areas — cancer, chronic inflammations and viral diseases — in which interferon could have widespread application.

Bache estimates that in the US 5.4 million cancer patients a year could receive interferon treatment. Assuming a five-

injection course of therapy at a factory price of \$10 per dose, this produces a \$270 million-a-year market. Subtracting \$100 million a year for competing drug therapies leaves an interferon market of \$170 million a year from cancer treatment.

For chronic inflammatory diseases such as arthritis, Bache estimates that interferon could capture half the market now occupied by steroid treatment. At 11.5 million patients and a course of five \$10 injections the market would be around \$300 million. Viral diseases are estimated to plague nine million people a year in the US. With a shorter course of three injections per treatment course sales would be worth \$270 million a year. Total US sales would then be around \$750 million.

Bache then estimates world wide sales on the basis of current pharmaceutical sales. In 1979, the US market accounted for 20% of the world market. Since new drugs take longer to penetrate non-US markets, Bache estimates non-US sales for interferon to be three times the US total.

The author of the report, Ronald Norman, formerly of Warner Lambert and Bache's specialist on the pharmaceutical industry, admits that some of the estimates are only guesses. In particular, the factory price of a dose could end up falling anywhere between \$1 and \$100 dollars a dose. "We create a rough guideline and leave it to our clients to make their own educated guesses" says Norman. (G.D. Searle in the UK has estimated that their fibroblast technique could bring the factory price down to around \$2 a dose.)

Joe Schwartz

United Kingdom

Coalite health survey talks

WORKERS exposed to the toxic chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) at Coalite and Chemical Products Ltd between 1968-1971 are to be told today the results of three surveys of their state of health (*Nature*, 6 March, page 2). But because of the make-up of the control group surveyed, interpretation of results is difficult, and the investigation may have to be repeated.

Officials of the Health and Safety Executive (HSE) are to meet the workers, and management, to give them the Executive's assessment of the surveys; but the Executive is expected to say that it is not possible to draw any firm conclusion from results so far.

Two of the studies — those on the blood chemistry and immunology — detected differences between the dioxin-exposed men and the control group; but the third study on chromosome changes reported no differences between the two. The study of blood chemistry showed changes in total blood cholesterol and high density lipoprotein cholesterol levels in the exposed workers. And the immunology

study reported reduced levels of two immunoglobulins, IgD and IgE in a significant proportion of them.

However, the control group used was not properly matched with the exposed group, making comparison between the two difficult. The control group included management staff undergoing lipid screening at the time.

One HSE official hinted that something might be salvaged from the studies when the raw data had been examined but admitted that the investigation may have to be repeated.

Even though the HSE persuaded Coalite to arrange the studies two years ago, it did not receive the results until a few weeks ago. This delay was due in part to a belief within the HSE that it lacked the necessary powers to compel Coalite to disclose the findings.

An Executive spokesman has now said that the HSE does have the legal powers under Section 27 of the 1974 Health and Safety at Work Act to serve a notice on any individual to disclose information which the Executive requires.

Alastair Hay

France

Cap la Hague restarted

THE accident at the reprocessing plant at Cap la Hague which resulted in the total loss of electric power a fortnight ago (*Nature*, 24 April, page 653) may have been the result of circuit overloading according to the CFDT, the main union representing nuclear workers at the plant. A spokesman for the union told *Nature* that some of the power supply equipment was 15 years old and had been initially designed for the reprocessing of low activity spent fuel only. However, high activity fuel was now reprocessed there although the plant had not been fully upgraded to cope with the increased current this demanded.

According to reports in the *Guardian*, Cogema decided to restart operations last week after receiving clearance from the Ministry of Industry. A spokesman for Cogema had said that radiation levels were acceptable. Workers at the plant, however, were worried that several parts of the plant were still highly contaminated. According to the CFDT spokesman, an expert who had visited the plant had recommended that it should not be restarted. The director however, decided to go ahead.

The union met at the end of last week to discuss how to react to Cogema's decision to restart operations even though

full repairs have not been carried out. In the absence of any detailed technical report, no agreement on what action to take was reached except that the union's concern over the decision should be expressed.

Cogema's desire to restart only eight days after the accident is seen as a move to reprocess spent fuel already stored at the plant to make room in the storage ponds for a consignment of Japanese spent fuel awaiting shipment in the UK. Workers believe that as soon as this backlog is cleared, the plant will be closed in two to three weeks time for full repairs. □

Mozambique

The politics of African archaeology

THE opening of a new museum at Manyikeni in Mozambique marks a change in the presentation of archaeology in Southern Africa.

At issue are the "Zimbabwes" — a chain of 150 ancient settlements characterised by central stone-walled enclosures spread across Mozambique and what is now called Zimbabwe. White settlers in Rhodesia argued that the stone walls were Egyptian temples built by the Queen of Sheba, while the Portuguese in Mozambique opted for their construction by mythical Lusitanians.

As early as 1905, the British Egyptologist, Randal McIver, established that not only were the ruins not Egyptian, but that they were built in the past 1000 years, and thus necessarily by local, black, people. Later all other archaeologists agreed. But that answer was totally unacceptable to the white settlers, who stuck by the Queen of Sheba.

The advent of scientific archaeology made this position increasingly untenable. The first carbon tests in the 1950s suggested that Great Zimbabwe, after which the country Zimbabwe has taken its name, was built around 1000 years ago. (Great Zimbabwe was the most striking of these settlements and with a population of 10,000, must have been the largest city in southern Africa at its peak in 1400.)

A guidebook to Great Zimbabwe published in the early 1960s contained the carbon dates and dismissed the Queen of Sheba explanations. In the late 1960s and early 1970s much better carbon dates were



Manyi Keni . . . first exhibition on basic data

produced for Great Zimbabwe, but the then Rhodesian government not only refused to permit them to be displayed, but ordered the removal of all dates from the Zimbabwe displays in museums. And the guidebook was allowed to go out of print. In 1975, when a new guidebook was being written, the government ruled that carbon dates could not be included.

In Mozambique the attitude was different. The Portuguese simply never excavated the Zimbabwes, so there was no data to contradict the Lusitanians.

In 1975, however, after independence in Mozambique one of the Zimbabwes — Manyikeni, 50 km west of Vilanculos was excavated. The work has been done by a Mozambican archaeologist, Joao Morais, and two British archaeologists, Peter Garlake and Paul Sinclair. Both Garlake and Sinclair had worked at Great Zimbabwe, and both quit over the suppression of scientific data.

Carbon dating, done free of charge by the University of Rome, shows that Manyikeni was occupied from 1250 to 1750, and that the walls were built around 1450. The stone walls, about two metres high, and similar to English dry stone walling, made an enclosure inside which about five families lived. The new museum displays show carbon dates and detail the

recent excavations and scientific stories.

Several scientific techniques have been brought to bear on the site, some for the first time. One is stratified sampling, in which the site was first divided into 20 metre by 20 metre squares, and then four randomly selected one metre by one metre trenches excavated within each of the larger squares. This gives a better overall picture of life in the settlement than just by digging at "likely looking" spots. Until the early 1970s, all the excavation was done inside the walls. But then it was realised that the bulk of the settlement was actually outside. The area outside is too large to excavate completely and stratified sampling is the only sensible way. Manyikeni is the first Zimbabwe to be extensively excavated outside the walls.

The excavation of this site represents one of Mozambique's first efforts to illuminate its history before the colonists arrived. It also reflects Mozambique's attempt to popularise science. The excavation was done by local people, under the supervision of the professional archaeologists. And the museum is primarily intended to teach local people their own history. Yet it also represents the first exhibition in Mozambique or Zimbabwe of some basic scientific data.

Joseph Hanlon

NEWS IN BRIEF

Three Mile Island health effects

HEALTH officials in Pennsylvania have discounted recent claims that increased infant mortality in the area around the Three Mile Island nuclear power plant was due to the accident at the plant last March. They point out that the area also experienced an increase in total births, and that the mortality rate therefore remained constant.

However, they have also reported a "surprising" degree of anxiety among residents living near the reactor, with significant increases in the use of tranquillisers and sleeping pills. Nearly half of 1,000 people interviewed in January who live within 10 miles of the plant reported physical symptoms of stress, ranging from headaches and diarrhoea to a loss of appetite and the appearance of rashes.

The survey, conducted by behavioural scientists at the Hershey Medical Center of Pennsylvania State University, also found that nearly 13% of those living within five miles of the Three Mile Island plant had become anti-nuclear activists. Dr. H. Arnold Muller, the State Secretary of Health, called this figure "very significant."

Drilling Project faces big cut

THE US House of Representatives Science and Technology committee has proposed that federal support for the first year of a massive Ocean Margin Drilling Project, to be sponsored jointly by the National Science Foundation and private oil companies, be reduced from \$5 million to \$500,000.

The \$5 million recommended by the Carter Administration in its revised budget proposals last month had already been reduced from \$10 million included in the original NSF budget proposal in January. However, the Senate Health and Human Resources Committee, voting last week on the NSF appropriations request for the fiscal year 1981, is proposing restoring funding for the project to about \$9 million — which provides room for an appropriate compromise.

MIT raises \$250 million

THE Massachusetts Institute of Technology announced last week that it has successfully raised \$250.2 million — \$25 million above its target figure — in a five-year fund raising campaign.

About a quarter of the total will go to endowing new faculty positions, and an

equivalent amount to new teaching and research facilities, including the construction of the new Whittaker College of Health Sciences. The remaining funds will go to supporting new programmes and other uses.

MIT is also expected to announce this week a major agreement with the Exxon oil company to support basic combustion research at the institute.



Chojecki held

MIROSLAV Chojecki (above), a young Polish physicist and human rights activist, was recently arrested under a "procurator's sanction", which empowers the police to hold him up to 90 days without charge or trial.

Chojecki was formerly a research assistant working for his PhD at the Warsaw Nuclear Research Institute. During 1976, he was a frequent observer at the trials in Radom of workers who had taken part in the June demonstrations against food price rises. In October 1976 he was dismissed from his post and has not since been able to find professional employment. He has recently been active in "Nowa", the "independent" (i.e. underground) dissident publishing group.

In an "open letter" written shortly before his arrest, Chojecki stated that since his first arrest on 30 September 1976, he has spent about five months in detention (equivalent to one day a week). His flat has been searched 15 times ("once a quarter") and his person 80 times ("once a fortnight"). On only three occasions was a search warrant issued.

Items confiscated from him range from a text book of Fortran programming to a jar of curry powder (thought by the police to be radioactive isotopes), cuttings from the official Polish press, three tins of imported meat and the contents of his waste-paper basket. He has been fined on a number of occasions for petty offences a total of 19,000 zł. (£350).

It is understood that he is likely to be charged in the near future with being in unlawful possession of printing equipment.

Burbridge to head astrophysics unit

DR Margaret Brubridge has been appointed to direct the new Centre for Astrophysics and Space Sciences at the University of California in San Diego. The centre has been organised to coordinate the scientific activities of several groups which carry out research in astrophysics and space science in three university departments — physics, electrical engineering and computer science. Also included in the regrouping of space science disciplines is the cosmochemistry group in the chemistry department. The centre will house under one roof high energy astrophysics, solar physics, optical and infrared astronomy, the Faint Object Spectrograph (Space Telescope) team, magnetospheric physics and radio astronomy. Laurence E. Peterson and Elden C. Whipple will be associate directors of the centre.

CND marches again

The UK Campaign for Nuclear Disarmament (CND) has organised a major demonstration to protest the installation of the Cruise missile on British soil. The demonstration will begin in Oxford and participants will march 18 miles to the US Air Force base in Upper Heyford, an expected site for the Cruise missile.

An internationally known organisation for the expression of grass roots opposition to nuclear war in the early 1960s, CND is undergoing a revival in the UK at the present time. "Judging from our mail and our discussions with the large number of volunteers that have come forward, people now are concerned about the international situation, particularly Carter's irresponsibility in Iran, the government's attempt to revive civil defence, and the increasing cost of the military budget at the expense of social services," says Caroline Coles of CND.

Monkey business

The Indian Commerce Minister, Z. R. Ansari, recently told Parliament that there was no proposal to lift the export-ban on rhesus monkeys, though several representations had been received from importing countries. The ban was imposed two years ago by the Janta Government following revelations that rhesus were being used in neutron bomb testing. The US drug industry is trying to convince the Indian government that the ban has adversely affected the introduction of several new drugs and even caused shortage of polio vaccine, which is sold in large quantities in India.

FEATURES



A social worker makes a health call on a Brazilian family . . . but will the bureaucratic machine get in her way?

Brazilian paralysis: the real disease

AFTER being invited to work in Brazil by the country's Minister of Health in January, received with official honours by the President of the Republic, and feted as a Nobel prize-winner (which he is not), Dr Albert Sabin, developer of the oral anti-polio vaccine, has left the country a disillusioned man. The muddle, inefficiency and sheer obstructiveness of the Brazilian Health Ministry proved too frustrating for Sabin to bear, and he has returned to his Charleston, Carolina, laboratory without taking leave of Health Minister Valdir Arcoverde.

"In truth," he commented, "it is difficult to take leave of a man who does not let himself be approached."

By Sabin's account, the history of Brazil's approach to its regular epidemics of polio is one of garbled statistics and

Health care in Brazil is not simply a matter of science and sound management. It is a matter of politics. **Maurice Bazin** reports on a case history — the 'Sabin episode'

official indifference to the plight of the thousands of children who still contract the disease. And he has said as much in a letter to President Figueiredo.

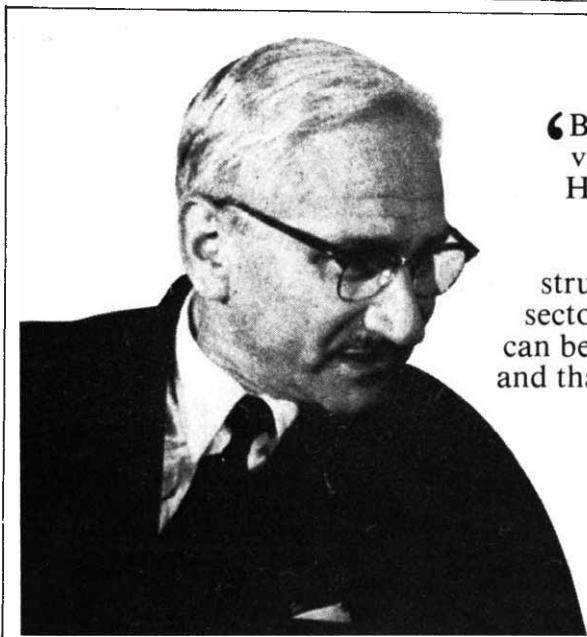
"Bureaucratic problems of various types do not permit that this work be properly developed," he complained. "I must emphasise to your Excellency that this

same bureaucracy was responsible for the failures in combating poliomyelitis in the past, and, as for the present, I have strong reasons to doubt that the next attempts will be successful."

The President has not replied.

The first major problem Sabin encountered during his two-month stay in Brazil was the impossibility he found in obtaining an estimate, however approximate, of the scale of the polio problem over the preceding ten years.

He pointed out to the Health Ministry that statistics published during that period by the Institute of Geography and Statistics (IBGE) on the one hand and the Health Ministry on the other differed by factors "at times as high as 60 and 170". Brazil's reports to the World Health Organization showed 15,000 cases of polio per year from



“Brazil needs a national vaccination campaign. However, this task will continue to come up against bureaucratic structures and unreliable sectors. There is a lot that can be done, must be done, and that is not being done”
— Albert Sabin

1969 to 1972 followed by an abrupt decline to 2,100 cases per year from 1973 up to the present. Could this effect be the reflection of a national immunization campaign carried out in 1971? Looking through the records, Sabin found out that the high figure came from the IBGE whose data was transmitted to WHO up to 1972, while the low figure came from the Health Ministry's own statistics. The apparent drop corresponded to no more than the switch in data sets. Furthermore, since 1975, IBGE had been forbidden to supply any more statistics about polio.

“Nobody lied or manipulated the data,” he said, “but the whole truth was not told. The officials of the Health Ministry are guilty of breach of duty because they did not inform the public about the change from one set of statistics to another. And still now they continue to give out improper information to WHO.”

While working with a local vaccination campaign Sabin heard that his proposal for obtaining realistic statistics about polio incidence from a survey of school children was considered by the ministry to be “of dubious statistical value”, although it had been approved by the minister six weeks earlier. But he organized a local statistical evaluation of the incidence of polio for 1969-72 and 1973-76 by looking for sequels of paralysis in children 10 to 11 years of age and six to seven years of age respectively in the Federal District around Brasilia today.

He enlisted the participation of local doctors and school personnel and found 57 cases of clear paralytic sequels in a sample of 25,000 children. From the detailed data, which represents a minimum evaluation, he could conclude that the IBGE statistics for these periods were closer to reality than those of the Health Ministry, which were at least five times below the true level.

Sabin's own data, however, are certainly still very much below a realistic estimate of the number of polio cases because they rely

upon a sample of children presently in schools. In the poor districts around Brasilia many children do not go to school, and physically maimed children are often beggars in the streets and not registered school children. The only technical comment that minister Arcoverde made after Sabin made his results public at the end of March was:

“Dr Sabin is an excellent epidemiologist, however, he is no a statistician.” To which Sabin replied publicly: “The Minister says that the ministry's statistics are satisfactory; I say it's completely untrue.”

In the course of his investigation in the Federal District, Sabin found out that 88% of the children had contracted polio before two years of age and 45% before reaching one year, and that parents only knew of it when they noticed that their child had difficulty in starting to walk. As he went to the slum satellite cities of the Federal District and around Brasilia, diagnosing the origin of children's physical deformities, he could remember that official statistics reported no polio cases outside the capital proper during 1976, 1977 and 1978. Today, he noted, the industrial suburbs of the city of Sao Paulo have a polio incidence five times higher than the city proper.

Using the weekly reports of polio cases for different states of Brazil, Sabin found that epidemics appeared to last much longer than in European countries, at times up to six months or more. However, looking with care at the geographical origin of the reported cases, he could prove that an epidemic propagates across a state and lasts the customary three to four weeks in each locality. He therefore proposed a plan of action to stop the ongoing epidemics in the state of Santa Catarina in the south of Brazil, which he had been called to help control in January. The state's health secretary mobilized thousands of school teachers, policemen and community members to carry out a massive vaccination

in one day in the communities immediately in front of the wave of contamination.

“We learnt that we could not do a vaccination in one day because the statistics about the number of children in the community were wrong. In some places we had vaccinated 110% of the children! But by the second day we reached almost all children, with teachers going door to door all day in the hot sun.” Three weeks later the last hospital admittance for polio was registered in Florianopolis, the state capital.

Open conflict between the scientist and the minister never occurred. Health bureaucrats, comfortably installed in their futuristic buildings in Brasilia, continued smiling to the scientist, but stopped answering his memoranda. In a mixture of efficiency and idealism, Sabin would announce in a memo that there were no steel lungs available to save children with affected respiratory muscles outside the cities of Rio and Sao Paulo, indicate how to recover the huge lungs which were used in the US before vaccination existed, and where to acquire new plastic models. He included in the memo the telephone numbers of the manufacturers.

“Unfortunately, these recommendations dissolved away in bureaucratic side tracts” he wrote in his open letter to the President of the Republic.

At the same time, the Society of Pediatrics of Brasilia declared that “vaccination campaigns are never executed adequately for administrative reasons mixed with politics.”

Sabin's letter to the President added: “Brazil needs a national vaccination campaign, organised in a highly efficient way and conducted every year. However, this task will continue to come up against bureaucratic structures and unreliable sectors. Just like in military operations, inexact information about enemy forces can bring about disasters; the same occurs when you face an epidemic disease . . . I have met in Brazil dedicated and competent men and women, capable of carrying through this task. I accompanied them in Goiás, Santa Catarina, Paraná and the Federal District, and I personally observed the contrast between their work and that of the official bureaucracy . . . There is a lot that can be done, must be done, and that is not being done.”

Despite Sabin's efforts the fundamental social problem remains of how to protect a whole population through a lasting organized effort of routine vaccination. To contribute to this effort was part of Sabin's plans for 1980. It is now very doubtful that anything will come of it.

For saying aloud what officials in power were not interested in hearing, Sabin is now back home, and his data on polio in Brazil will be published in English-speaking professional magazines only. The president of the Society of Medicine and Surgery of Rio has compared the “Sabin episode” to the case of Galileo. □

CORRESPONDENCE

Expanding on Parkinson's Law

SIR, — Parkinson summarised his large experience of administration in the law that Work Expands So As To Fill The Time Available For Its Completion (C.N. Parkinson, *Parkinson's Law*, 1958). A corollary of this law, that the proportion of administrators within a public organization tends to increase with the size of that organization, has been confirmed by empirical observation (R. Moss, *Nature*, 273, page 184, 1978). Schumacher in *Small is Beautiful* (1974), challenged conventional wisdom by showing that large, centralized organizations are often less efficient than small ones, and Moss made observations on the staffing structure of the Natural Environment Research Council (NERC) which showed that component bodies of NERC in which the administration was dispersed amongst many addresses have fewer administrators and other support staff than other, more centralized bodies of comparable size. Further, J. Flux's detailed study of the Ecology Division of the New Zealand Department of Scientific and Industrial Research (in *Focus on Social Responsibility in Science*, 1979), showed that the production of scientific papers per man decreased as the organization increased in size.

These scientific observations have not been followed by a reorganization in the administration of scientific research. Colleagues have suggested that this is partly because the original empirical observations were not accompanied by a theoretical model. Such a model is therefore presented.

Consider the situation in which we have a number W ($W = 4$ in the example of the figure) of individuals or groups of individuals doing a certain amount of work such as making scientific discoveries or collecting tax. The simplest possible situation is that each individual worker gets on with his job, pausing occasionally to speak with colleagues, answer letters and arrange for the office windows to be cleaned. As W increases, such primitive liaison techniques may become less effective and an administrator may be appointed.

In the model, the administrative workload of an individual is a function of the number of people with whom he communicates. This is the number of lines of communication l_i , as may be seen in the figure and, for brevity, is subsequently referred to as 'workload'. In stage one of the model each speaks with each of his colleagues and also with the outside world

$$l_{wi} = W$$

where l_{wi} is the workload of worker i .

In stage two of the model, when the administrator is newly appointed, each worker continued to speak with other workers, but also communicates with the administrator. Of course a 'worker' in this model could as easily be a constituent organization within an 'umbrella' organization such as the Agricultural Research Council (ARC), the Medical Research Council (MRC) or NERC. In stage two

$$l_{ai} = W + 1$$

and, as before

$$l_{wi} = W$$

where l_{ai} is the workload of administrator i (of course the suffix i is redundant at this stage, but it becomes useful later).

When the excitement of reorganization has settled into the humdrum of routine, the administrator has an idea which many readers will recognise. "It is grossly inefficient for all of you (W) to be wasting time liaising both with each other and with me (a_i). Let us reorganize our lines of communication so that you all communicate through me". This is

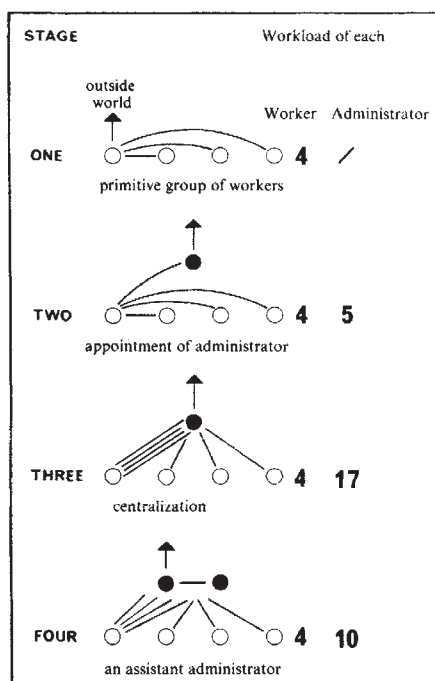


Figure. Illustrating the number of lines of communication (administrative workload) between members of an organization containing four workers, as it passes through the four stages of Parkinson's Progression.

Note. For clarity, lines of communication for one worker only are shown — lines of communication for the others follow an identical pattern.

stage three in the figure. "That will be much more efficient". Efficiency, of course, is only one objective of this course of action. Lust for power is an important motivation and the concept of 'accountability' is often pressed into use at this stage.

In stage three the workload of each worker remains the same, although cooperation amongst them is slowed down by the length of time that their messages lie on the administrator's desk. But the workload of the hard-pressed administrator now rises sharply as he grapples with all the matters that had previously been the subject of direct communication between workers. As well as communicating directly with W and the outside world, he now passes minutes to and from all the W .

$$l_{ai} = W^2 + 1$$

and, as before

$$l_{wi} = W$$

This situation is intolerable: in our example it can be seen that the administrator's workload has increased by more than three times, without any increase in the number of workers. This is the effect of centralization. In desperation our administrator applies for assistance. This is stage four where efficient co-operation amongst administrators gives

$$l_{ai} = \frac{W^2}{A} + A$$

and, as before

$$l_{wi} = W^2 + A$$

A being the number of administrators. Our exemplary administrator in the figure has thriftily indented for only one assistant and so the number of administrators has now doubled, but the workload of each is

nonetheless double what it was in stage two. Both administrators are working very hard, as evidenced by the large number of minutes processed since stage four came into operation. More assistants may well be needed to cope with the increased workload.

Our two administrators may well assume that the more colleagues they have, the smaller will be their individual workload. This is true up to a point, the point being four administrators to four workers in our example. Beyond that number, however, l_{ai} increases again as the administrators spend an increasing proportion of their time sending minutes to each other. In general, the minimum workload per administrator $l_{ai\min}$ is attained when $A = W$, that is when the 'maximum ratio' referred to by Parkinson, beyond which the proportion of administrators is unlikely to increase. It is notable that the maximum percentage of non-scientific staff in any of the component bodies of NERC is exactly 50%.

At this point, let us turn our attention to the next rank up in the hierarchy, the chief administrator c who appointed our administrators in the first place and to whom they are responsible. His workload will increase as A increases. Initially, his workload will bear the same relation to A as that of A did to W . But, as A increases, he may decide that further centralization is called for in pursuit of increased efficiency... with the inevitable consequence that stages three and four repeat themselves at this and possibly higher levels in the hierarchy.

Parkinson's Law has long been regarded as a joke by administrators who know that they work very hard. But what do they work hard at? The writer's experience is that questions such as this, pursued in the course of objective scientific analysis, are sometimes resented by administrators of scientific organizations. The model presented here shows simply and not unrealistically that the effects of centralization are to increase the amount of information that an administrator processes, whilst decreasing the proportion of workers in the organization. Had this model been available and accepted by those in authority some years ago, the reorganization of, for example, local government and the National Health Service, accompanied by decreased standards of service and increased administrative staffs, might not have occurred.

A problem with the model is the simplified assumption that all lines of communication are equivalent in the amount of work that they represent. A logical step next is to test the robustness of this approximation by making measurements of the amount of work per line of communication in the field, factory, office or laboratory.

The principles that the number of administrators tends to increase irrespective of the amount of external work done by an organization, due to Parkinson, and that increased centralization is accompanied by decreased effectiveness, pointed out by Schumacher², were empirical and inductive observations made by wise and experienced men. I provide a deductive and quantitative basis for these classic observations, a step towards management by reason rather than intuition.

Yours faithfully,

ROBERT MOSS

Station House, Crathes,
by Banchory Kincardineshire,
Scotland.

NEWS AND VIEWS

The third category of extraterrestrial material

from A.E. Fallick and C.T. Pillinger.

OVER the past ten years two new sources of extraterrestrial material have led to rapid advances in our understanding of the chemistry of the cosmos. The first, of course, was the return of samples from the Moon by the Apollo and Luna spacecraft. The second was the discovery of early condensate-type inclusions in the Allende meteorite, which were found to have anomalous isotopic abundances of various elements and are believed by some workers (see *Nature* **283**, 813; 1980) to be older than the Solar System itself.

Now a third class of extraterrestrial material, consisting of the "Brownlee particles" collected in the stratosphere by specially adapted U-2 aircraft, and meteor ablation debris scavenged from the ocean floor (see *Nature* **279**, 20; 1979), may be added to the above. As common meteors have orbital parameters indicative of a cometary origin, the new material is of particular interest because it is generally held that comets represent the most primitive sample of the Solar System. Some arguments (for example Napier & Clube *Nature* **282**, 455; 1979) have been presented that comets may even be planetesimals temporarily captured during passage of the solar system through the spiral arms of the Galaxy. If this view is correct cometary material becomes of immense importance, for it is then possible that it may be rapidly cooled, explosive debris from highly evolved, massive stars.

Last year, Ganapathy and Brownlee (*Science* **206**, 1075; 1979) definitely identified two stratospheric particles as micrometeorites by comparing volatile and nonvolatile trace element concentrations with those of CI chondrites, reckoned also to be primitive solar system material. Studies on single grains less than about 40 μm in diameter are not easy but results reported at the recent Lunar and Planetary Science Conference* show studies of stratospheric and deep sea material have great potential. Don Brownlee and colleagues (Seattle and Caltech) have recognised unmelted carbonaceous chondrite, CI and CM materials in deep-sea sediments, and P. Fraundorf and co-workers (St. Louis) have demonstrated the presence of the "cosmic" 10 μm feature in the optical absorption spectrum of

stratospheric particles. B. Hudson and co-workers also of St. Louis, succeeded in refining experimental techniques to allow them to determine He, Ne and Ar concentrations in a sample of thirteen Brownlee particles, each typically 10 μm across and with estimated total weight of around 10^{-8} g. They found a $^{20}\text{Ne}/^{36}\text{Ar}$ ratio of 9 ± 3 , which resembles the ratio for lunar soil fines (~ 7) more than it does the terrestrial ratio (~ 0.5). Assuming the gases to be solar in origin, the time taken to accumulate the Ne and Ar could be the surprisingly low figure of 10-100 years, based on current estimates of solar wind fluxes. As microparticle analysis seems likely to become more efficient and with proposals for a European Space Agency mission to the comet Halley in 1985-6 still active, perhaps the 1980's will be the decade of cometary science.

Work on the first two categories of extraterrestrial materials continues to become much more refined. New studies of solar noble gases in lunar regolith minerals were unveiled by Peter Signer (Zurich). His group have measured ^4He or ^{36}Ar and ^{38}Ar in some 90 individual plagioclase grains and failed to find evidence for whole grain saturation in either ^4He or ^{36}Ar . A search for microscopic saturation is under way using track density studies; the absence of such saturation as a common feature would allow mean noble gas abundances in the ancient solar wind to be inferred from minerals exposed at the appropriate time. M. Thiemans and R. N. Clayton (Chicago) are studying $^{15}\text{N}/^{14}\text{N}$ in the Apollo 17 deep drill core in an attempt to reconstruct the history of the supposed solar wind nitrogen isotope variations. "Nitrogen is the newest member of the family of noble gases" quipped Clayton.

Dieter Heymann (Rice, Texas) attempted to develop a plausible Xe isotope stratigraphy for a massive star immediately prior to, and after, the supernova explosion. Because of the paucity of observational constraints such a model is difficult to assess, but Heymann pointed out the remarkable agreement between the Xe isotopic profile of the explosive products and the well-defined Xe components in carbonaceous chondrites. More stringent testing will come from the application of the model to isotope profiles of other elements.

Sam Epstein (Caltech) has devised a technique whereby $\delta^{17}\text{O}$ may be estimated to $\pm 0.5\%$ from $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ determinations on CO_2 . This obviates the necessity for running O_2 in the mass spectrometer. The procedure was checked by analysing three inclusions from the Allende meteorite and then applied to investigating reactions involving excited species of oxygen compounds. G. Arrhenius and others have suggested that similar reactions may produce strongly nonlinear kinetic isotope fractionations (see *Nature* **279**, 20; 1979); however, in his preliminary experiments, Epstein found no evidence of this, despite a spread of 100% in $\delta^{18}\text{O}$. An important step forward in understanding the stable isotopes of H, C and N in extracts from meteorites was reported by F. Robert and colleagues, also from Epstein's laboratory at Caltech. Using a combination of solvent extraction, HF hydrolysis and stepwise heating techniques, they detected isotopic differences in C and N between different organic components in CM chondrites. Deuterium associated with insoluble organic fractions is enriched by 90% (sic) compared to inorganic nitrogen, and decoupling from ^{13}C and ^{15}N enrichment is suggested. Interest now centres on whether equilibrium-type mechanisms of Fischer-Tropsch-type reactions will be successful in accounting for such observations, or if (as seems more likely) different source materials for the various components are required.

Nature still continues to spring surprises on those investigating the host phases of rare gases in meteorites. The Chicago-Berkeley controversy over the identification of the various carriers took a new twist with the report by Roy Lewis and co-workers (Chicago) of no fewer than five different forms of carbyne in Murchison and Allende. Carbynes are a series of linear polymorphs of carbon with alternating single and triple bonds; they are the stable low pressure forms above 2600°K and have recently been suggested by Webster (*Mon. Not. Roy. Astron. Soc.* in press) as candidates for interstellar dust and stellar

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*The XI Lunar and Planetary Science Conference was held at the NASA Johnson Space Centre, Houston, Texas, March 17-21, 1980.

condensates. U. Ott (Berkeley) has made a detailed study of colloidal separates from an Allende acid residue with the aim of unravelling more clues to the enigmatic phase Q (defined operationally as a residue fraction soluble in oxidising acids). His results are consistent with Q being carbonaceous rather than an inorganic

mineral. Several years ago, Srinivasan *et al.* (*J. geophys. Res.* **82**, 762; 1977) compared Reynolds' first measurements on xenon in meteorites to the opening of Pandora's box: perhaps the pace of advance now encourages us to believe that before long the reluctant Hope will be coaxed out of the box also. □

Discrete Σ -hypernuclear states

from R. H. Dalitz

LAST summer, an experimental group headed by B. Povh, Max-Planck-Institut für Kernphysik, Heidelberg and working at CERN, reported to a conference held at Vancouver, the observation of well-defined peaks in the pion spectra following the $K^- \rightarrow \pi^\pm$ reactions at 0° , for K^- mesons of momentum 720 MeV/c incident on ^7Li , ^9Be and ^{12}C targets. These peaks are in the part of the spectrum which corresponds to the production of a Σ -hyperon, and the widths reported for these discrete states are of order 10 MeV. These narrow Σ -hypernuclear states were not anticipated by theoreticians but turned up in the course of a study of the highly-excited Λ -hypernuclear states produced in the $K^- \rightarrow \pi^-$ reaction.

The hyperons Λ and $(\Sigma^0, \Sigma^+, \Sigma^-)$ are well-known. The Σ -hyperons are about 75 MeV heavier than the Λ hyperon and have the same strangeness quantum number, $s = -1$. $\Sigma \rightarrow \Lambda$ reactions, of the type $\Sigma^- + p \rightarrow \Lambda + n$, are exothermic and allowed by all the selection rules governing strong nuclear interaction processes. This led to the general expectation that a Σ -hyperon in contact with a nucleus would transform to a Λ -hyperon in a time of order 10^{-24}s . Since this is less than one-tenth of the time taken by a hyperon to travel across a nucleus, it appeared most unlikely that discrete quantum states could exist for a Σ -hyperon within a nucleus. All of our experience of Σ -hyperon processes observed in nuclear emulsion and in bubble chambers was consistent with this view. For example, the study of X-rays emitted by Σ^- -hyperons in atomic orbits around nuclei showed that Σ^- -hyperons coming to rest in condensed matter are captured by the nucleus predominantly from high-lying orbits. As the Σ^- -nucleus system cascades down through its atomic levels (which are due to their Coulomb attraction), emitting a photoelectron or X-ray at each step, the capture reaction $\Sigma N \rightarrow \Lambda N$ occurs as soon as there is any appreciable overlap of the Σ^- wavefunction with that of the outer nucleons of the nucleus. Thus, the nuclear capture processes take place on the extreme periphery of the nucleus, and the yield of X-rays due to transitions between the inner atomic orbitals is low relative to those for

the outer orbitals since few Σ^- hyperons escape nuclear capture and reach the inner levels.

The $K^- \rightarrow \pi^\pm$ reactions studied in these CERN experiments are termed "strangeness exchange reactions". The strangeness of the meson increases by $\Delta s = +1$, and the strangeness of the target nucleus (initially $s = 0$) becomes -1 , since total strangeness is conserved by the strong interactions. Thus the final nuclear system reached includes one hyperon. These reactions have a special feature, due to the fact that the mass difference $(m_K - m_\pi) \approx 355$ MeV is greater than the mass difference $(M_\Sigma - M_N) \approx 255$ MeV. In this case, the $N \rightarrow \Sigma$ strangeness exchange reaction can take place for a K^- meson at rest and in contact with nuclear matter. For non-zero incident momentum p_K , we confine attention here to pions emitted at 0° to the K^- momentum since this configuration gives the least momentum transfer q to the target nuclear system. As p_K increases from zero, this momentum transfer falls until a "magic momentum" p_0 is reached at which the momentum transfer q is zero. As p_K rises further, the momentum transfer q increases from zero, but it rises only to 290 MeV/c, even in the limit of infinite incident momentum.

For the 0° reaction, the strangeness exchange process allows no spin flip for the target nucleons. At the magic momentum $p_0 \approx 280$ MeV/c, the orbital states in the initial nucleus are preserved in the final hypernuclear state, since the momentum transfer $q = 0$. The reaction then consists of replacing a nucleon by a Σ particle, with precisely the same wavefunction; the amplitude for this replacement receives coherent contributions from all the nucleons of the target and the cross section is correspondingly enhanced. There has already been much experience with these coherent transitions for the case $N \rightarrow \Lambda$, where $p_0 = 530$ MeV/c (Povh *Ann. Revs. Nucl. Phys.* **28**, 1; 1978). In practice, it has become evident that this coherence extends only to the nucleons in a given shell, but the coherent transitions leading to final Λ -hypernuclear states with the same spin-parity J^P as the target nucleus do stand out clearly in the 0° pion spectrum, even for incident K^- momentum $p_K = 720$ MeV/c, for which we have $q \approx 40$ MeV/c. For p-shell nuclei, which have had the most

detailed study, the Λ -hypernuclear states reached in this way are necessarily excited states. Substitution of a $1s$ neutron by a Λ hyperon leaves a hole in the $1s$ neutron shell; substitution of a $1p$ neutron by a $1p$ Λ hyperon leaves the V particle far above its $1s$ ground state. These remarks also apply to the Σ -hypernuclear states coherently produced by the strangeness exchange transition $N \rightarrow \Sigma$. The important difference was the expectation that the strong absorptive reaction $\Sigma N \rightarrow \Lambda N$ would cause these states to be so broad as to be unobservable in the pion spectrum. The CERN experiments have now shown that this expectation does not hold for all Σ -hypernuclear levels; some discrete levels do exist, even if only for a time of order 10^{-22}sec .

First, it is useful to remark that some particular Σ -hypernuclear states are expected to be semi-stable, i.e. to have lifetimes of order 10^{-11}sec , comparable with the lifetime for free Σ^- hyperons, if they exist as bound states. The clearest examples are the systems $(\Sigma^- + \text{neutrons})$; charge conservation does not allow any transition $\Sigma^- \rightarrow \Lambda$ on a neutron since there is no nucleon state with negative charge. Despite much searching, no empirical evidence has been found for any bound states of this type, nor for the charge-symmetric systems $(\Sigma^+ + \text{protons})$.

Gal and Dover (*Phys. Rev. Lett.* **44**, 379; 1980) have now made calculations intended to demonstrate that the existence of some discrete Σ -hypernuclear states is compatible with our other knowledge of hyperon-nucleon interactions, and to identify the structure of these states. The strength of $\Sigma N \rightarrow \Lambda N$ absorption is taken from experimental data, as interpreted in terms of theoretical hyperon-nucleon potentials. The rate used is $(v\sigma_{\text{abs}}) \approx 19$ mb, the average being over the nucleon momentum distribution in a nucleus, v denoting the Σ -N relative velocity. The Σ -nucleus interaction can also be viewed as a "cloudy crystal ball", i.e. a potential with the nuclear shape $\rho(r)$ and a complex strength $(U + iV)$. Then V is proportional to $(v\sigma_{\text{abs}})$ and therefore known. U has to be left as a free parameter, whose value can be determined later from the energy observed for an identified Σ -hypernuclear level, but it is not of importance for the total absorption rate. The solution of the Schrödinger equation for the Σ -hyperon in this potential will give a complex energy value $E = (\epsilon - i\Gamma/2)$ for each orbital (n, l) , the imaginary part $\Gamma(n, l)$ being the width for this orbital.

For heavy nuclei, the Σ hyperon sees "nuclear matter" and the width Γ does not depend strongly on the orbital (n, l) , when this lies dominantly within the nucleus. Typical values for Γ are then in the range 20–30 MeV, a width large relative to the spacing of the energy levels so that the individual levels cannot be distinguished.

For light nuclei (^{12}C and lighter, say), it is

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important to take into account the isospin- and spin-dependence of the ΣN interaction. For ΣN isospin 3/2, there is no absorption possible, since the ΛN state has isospin 1/2; the absence of nuclear absorption in a state ($\Sigma^- +$ neutrons) is a particular example of this situation. Also, we believe that, for low ΣN energies, the $\Sigma N \rightarrow \Lambda N$ absorption rate is much less in the singlet spin state than in the triplet spin state. Hence the observation of a narrow Σ -hypernuclear width calls for a configuration where (i) the nucleon density is small where the Λ -particle is, (ii) the isospin-3/2 ΣN interactions have a high relative weighting, and (iii) the singlet ΣN interactions have a high relative weighting, and (iv) its formation is coherent. For ^{12}C target, this state is identified by Dover and Gal to be the isospin-3/2 particle-hole state with structure $((1p)_{N^{-1}}(1p)_{\Sigma})_{J=0^+}$, its calculated width due to absorption being about 5 MeV. The isospin 3/2 state can be formed in both reactions $K^- \rightarrow \pi^{\pm}$, and this is also the case empirically. No other state considered has width much less than 20 MeV and no others are claimed empirically, at present. The isospin-1/2 state with the same structure has a calculated absorption width of about 18 MeV.

Two Σ -hypernuclear peaks were seen for ^9Be target. The lower state has only one possible interpretation, with a calculated absorptive width of about 7 MeV, consisting of the Σ -hyperon attached to the low-lying spinless isospin-0 states of ^8Be . The outstanding candidate for the upper peak is the isospin-2 configuration built on the isospin-1 ^3P core occurring at about 17 MeV in ^8Be , for which the calculations give $\Gamma = 6$ MeV, but there are

a number of other $A=9$ Σ -hypernuclear states with predicted widths of less than 12 MeV.

When these interpretations have been tested in more detail and verified, the properties of these exceptional states will give us information about the ΣN interactions otherwise difficult to obtain. First, their absolute location will determine the mean Σ well depth U . The spacing between the two ^9Be peaks will give another measure of this real part of the ΣN potential. The most interesting situation will be that for target ^{16}O , which contains both $p_{3/2}$ and $p_{1/2}$ nucleons. Two particle-hole states exist, which can both be produced coherently; their wave-functions are linear superpositions of the two configurations $((1p)_{N^{-1}}(1p)_{\Sigma})_{J=0^+}$, for $j=3/2$ and $1/2$. The measurement of their spacing will give us a measure of the ΣN spin-orbit interaction, just as we have recently learned the strength of the ΛN spin-orbit interaction from the spacing between the corresponding $^{16}\text{O}^*$ states (Brückner et al., *Phys. Lett.*, **79B**, 157; 1978). With data having less background, it may be possible to see the Σ -hypernuclear spectrum in more detail and to learn empirically about the spin-spin and isospin-isospin components of the hyperon-nucleon forces. An improved K^- beam is now being installed at CERN to allow these Σ -hypernuclear experiments to be carried out for lower incident momenta p_K , closer to the magic momentum $p_0(\Sigma) \approx 280$ MeV/c, so that the coherent states with narrow width will stand out more strongly in the data. We may expect more information on the Σ -nucleus interaction from this experimental program during 1981. $\square$

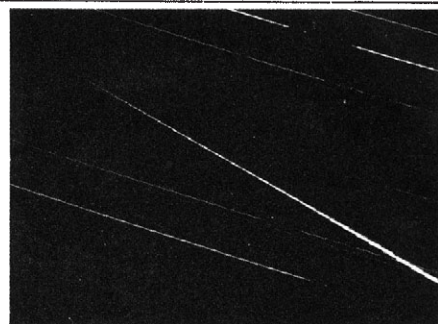
Meteoritic fireballs and luminous efficiency

from David W. Hughes

LUMINOUS streaks of light flashing across the sky are caused by cosmic dust particles burning out in our atmosphere. They are a common enough phenomena and have been seen ever since man looked up to the heavens, but even to-day they still present problems. One of the more pressing ones is the discovery of the exact relationship between the light intensity of the trail and the mass and velocity of the incident object. Things are relatively simple if the incident particle is less than about 1 cm in radius. These small meteoroids burn out at heights between about 80 and 110 km, in an atmospheric region where the mean free paths of the molecules are much larger than the meteoroid dimensions. The atoms that ablate from the particle leave without serious mutual interference. They have

kinetic energies of a few tens to some hundreds of electron volts and are thus excited and ionized. Also they can excite and ionize the molecules they collide with. Excited atoms and molecules emit radiation and the luminous power so produced has been found to be proportional to the rate of loss of kinetic energy of the ablated atoms. The constant of proportionality, known as the luminous efficiency factor, depends on the velocity of the incident meteorite, its composition (stone, iron, stoney iron) and its physical structure (i.e. whether it is a solid body or a dust ball).

Most of the early work in this field was done by Öpik and he prepared an indispensable series of tables which enabled the initial masses of small meteoroids to be estimated from measurements of the meteor luminosity and the meteoroid velocity.



The Pribram fireball. A rotating shutter cut off the light ten times a second. The number of breaks in the trail shows the meteor took seven seconds to cross the photograph. (Ondřejov Observatory, Czechoslovakia)

The problem becomes much more difficult in the case of the large meteorites. These lose the majority of their mass in the lower atmosphere where the mean free path of the air molecules is very much smaller than the size of the incoming object. So a large aircap is formed around the meteorite, shock waves occur in the region between this aircap and the atmosphere it is moving into and the meteorite is followed by an active, turbulent wake. Visual radiation is usually emitted from all these regions as well as from the meteorite itself. There is also another complication. Observations of meteors using Super Schmidt cameras have shown that the small meteoroids do not decelerate appreciably in the atmosphere. The loss of energy they suffer is exhibited by a mass loss rather than a velocity loss. The observed radiation also comes in the main from the ablated meteoroid atoms. The large meteorites however do decelerate. In many cases they are retarded to the free fall velocity and the remnants of the meteorite drops to the Earth's surface. Radiation also comes from many sources.

The luminous efficiency of meteoritic fireballs has been discussed in detail in a recent paper (*J. geophys. Res.* **84**, 6255; 1979) by Douglas ReVelle of Northern Arizona University and R. S. Rajan of the Carnegie Institution of Washington.

The authors stress the fact that the majority of the radiation from the fireball, when it is low down in the atmosphere, is in the ultraviolet region of the spectrum. They also take into account the deceleration term for the first time. Their theoretical work can unfortunately only be checked by reference to three meteorite entries. The first is the Pribram meteorite which fell in Czechoslovakia in 1959. The fall was photographed by a series of all sky camera's and the authors estimate that about 53 kg of meteorite eventually hit the earth even though outside the atmosphere the meteorite had a mass of around 1300 kg. — a loss of 96% of the mass. The other two meteorites, Lost City and Innisfree fell and were photographed in North America. In both cases about 70% of the initial mass was lost as the object came through the atmosphere. These mass loss figures are supported by an analysis of the cosmic ray tracks in the recovered meteorites. The

bombardment of the meteorite in space by cosmic rays produces a network of radiation damaged material. The density of this network shows appreciable depth dependence and a measurement of this density in the recovered meteorite leads to an estimate of how much material overlaid that region when it was in space.

The photographic records of these meteorites are of paramount importance because from them the velocity of the meteorite and the luminosity of the fireballs may be calculated as a function of height. ReVelle and Rajan applied their theoretical model to the observed light and velocity profiles and, after introducing a few assumptions (such as that the radiation field was isotropic and that the mass of the main body at any specific moment is much larger than the mass of the associated fragments), they obtained a curve which showed how the luminous efficiency varies as a function of height in the atmosphere and as a function of meteorite velocity.

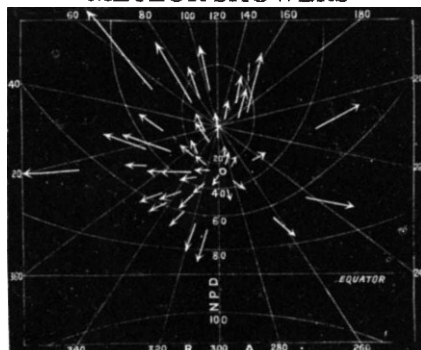
The authors compared their results with a low velocity laboratory simulation experiment carried out by Givens and Page (*J. geophys. Res.* 76, 1039; 1971). Here artificial meteors were produced by firing 0.6 mm stainless steel spheres with a light gas gun into a column of still air at 0.052 atmospheres of pressure. The resulting 'meteor' train was observed using a series of broad band multiplier phototube radiometers. The velocities of the spheres were about 8 km s^{-1} in comparison to the Pribram meteorite which initially had a velocity of 21 km s^{-1} and Lost City and Innisfree meteorites which had velocities about 14 km s^{-1} . When corrections were made for the velocity effect the results agreed very well.

The main conclusion of ReVelle and Rajan is that there is a phase lag between the ablation of meteoritic material and the emission of luminosity. The luminous efficiency was found to be a rather complicated function of the meteorite velocity. Also when the meteorites Lost City and Innisfree were losing mass at the maximum rate, the luminous efficiency in the visible wavelength region of the spectrum was about 0.3%. For the faster Pribram meteorite the value was about 0.04%. The authors adding the proviso that the Pribram results are regarded as being less reliable.

It is important to note that the 0.3% result is a factor of between 5 and 10 higher than the empirical luminous efficiency assumed by Cepelcha and McCrosky (*J. geophys. Res.* 81, 6257; 1976) in their analysis of fireball trails. This means that even though only 3 parts in 1000 of the energy lost by a meteorite goes into visual radiation this is considerably more than was estimated previously. So, bright fireballs are now thought to be produced by smaller incident meteoroids and the total mass of cosmic material hitting the Earth in the fireball size range has to be revised downwards by a factor of 5 to 10. □



100 years ago METEOR SHOWERS



Shower of Aquariads July 27-30th.

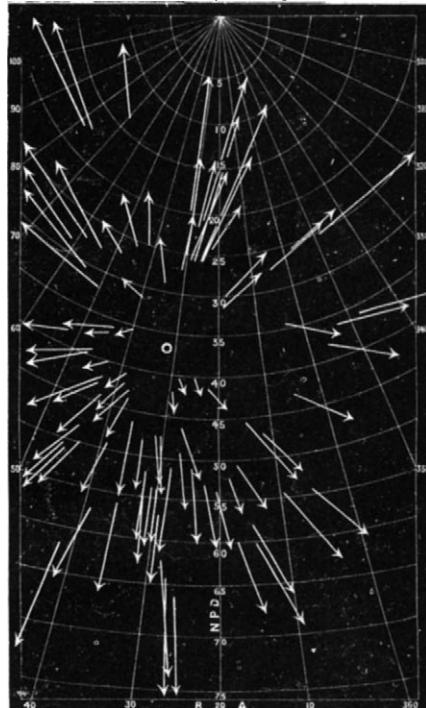
SEVERAL of the meteor streams observed at Bristol within the last two years appear to be of such marked intensity as to merit special description, and the following notes in connection with them may possess some interest to observers.

My observations in July, 1878, led me to recognise a large number of slow meteors with long paths (averaging 17°) and occasionally leaving faint trails of sparks, ascending amongst the stars of Pegasus, Andromeda, &c., which, by their parallelism of motion, obviously proceeded from a common radiant point south of Pegasus. I watched them narrowly, and determined the position with fair precision as near δ Aquarii.

A succession of clear nights occurred from July 26 to August 2 in 1878, and I obtained some lengthy observations. In about twenty-

two hours of watching more than 400 shooting stars were seen in the eastern sky, chiefly amongst the constellations of Perseus, Cassiopeia, and Andromeda. I saw many swift meteors leaving short streaks and otherwise exhibiting much uniformity in their appearances and directions. The radiant point was not reconcilable with that of the well-known annual shower of Perseids. It was sharply defined about 3° S. of the group χ Persei, and the maximum of the shower was witnessed on July 31, when 21 meteors were noted diverging from the point described.

Shower of Perseids II July 28-August 1st.



From *Nature* 21, 29 April, 621; 1880.

The Uninvited Guests

from Graham Darby

MOST people are no doubt unaware that they are accompanied through life by several herpes viruses lying dormant in the tissues of their bodies. The human herpes viruses have the characteristic of establishing long-term relationships with their hosts, emerging only infrequently to cause disease.

Since these viruses are ubiquitous in the population the first encounter between the infectious agent and its human host usually occurs early in life and results in an inapparent infection or rather mild disease. In the developed countries of the world this initial encounter often occurs later in life and, paradoxically, this delay may result in a more serious primary disease. Subsequently the viruses remain with us for life. There is little evidence that cytomegalovirus (CMV) or Epstein-Barr virus (EBV) usually cause further problems but both herpes simplex (HSV) and varicella-zoster (VSV) which establish latent infections in the peripheral nervous system may re-

emerge to cause recurrent episodes of disease. HSV type 1 causes periodic eruptions of cold sores around the mouth but these generally heal quickly and leave no scars. However, a closely related virus, HSV type 2, is more damaging as it usually infects the genital regions where it causes a painful and often socially embarrassing disease. Indeed, in some areas of the world it is emerging as the most common problem seen in V.D. clinics. VZV can remain latent for many years after childhood chicken pox but it too many reactivate, usually late in life, to cause shingles.

An overall view of our understanding of these agents and the diseases caused by them was provided by a meeting in Atlanta this Spring*. Both virologists and clinicians attended the meeting and the growing clinical importance of molecular biology was nicely demonstrated by B. Roizman

*An International Conference on Human Herpesviruses was held at Emory University, Atlanta, Georgia, U.S.A. from March 17th — 21st, 1980.

Deep sea diving

from M. J. Halsey

A new record was recently set at Duke University when three men successfully underwent conditions simulating a dive to a depth of 650m of sea water. This achievement set the background for a recent workshop* on the problems of very deep dives, in the range 460-760m (1500-2500ft).

Fifty years ago men dived using only compressed air but nitrogen narcosis, which increases with increasing partial pressure of the gas, effectively prevented this technique from being used at depth. The subsequent development of helium/oxygen mixtures allowed safe diving to depths beyond 50m, but problems develop when the 450-550m range is reached. At these depths the high pressure neurological syndrome (HPNS), characterised by muscular tremor, disorientation, and short sleep-like periods of loss of attention develops; in animal experiments even higher pressures have been shown to cause convulsions. However, a breakthrough has now been achieved by the discovery that HPNS can be overcome by the use of low partial pressures of anaesthetics. It is this that has led to new techniques and to new records in deep diving. In experiments with animals, gases with anaesthetic action such as nitrogen or nitrous oxide and low doses of intravenous anaesthetics such as ketamine or althesin have been found effective. Nitrogen deliberately introduced in small quantities into the helium/oxygen breathing mixture was the basis of the trimix which was used in the record chamber dive. This has also been used in the successful open sea dive to 460 m by Comex, S.A. Marseille, which has set a new standard for deep

water work.

Current work on a wider pharmacological approach to the problem suggests that selective drugs for HPNS may exist. Structural isomers of some steroid anaesthetics, such as althesin, have been found in animals to continue their action against HPNS even though they no longer have anaesthetic effect.

Additional problems in deep diving include those of temperature control, respiration and decompression. Temperature control is of great concern in working dives because high pressure seems to affect the body's thermoregulatory capacity. Not only is there a narrowing of the limits within which the body can make corrections to maintain a stable temperature but also the subjective experience of heat and cold may become divorced from actual conditions.

Respiration in chamber dives is not a general problem but dyspnoea, exacerbated by exercise, is present. This could become a limitation in future deep dives. The phenomenon appears to be unrelated to gas density but is an aspect of HPNS that is not alleviated by nitrogen. Decompression from deep depths is proving possible although recompression or a change of gas breathing mixture may prove difficult.

Although diving beyond 460 m has now certainly been made possible, and both naval and commercial interests agree there will be a definite need for such dives, it must be remembered that the principles underlying the effects described above are not at all understood. The number of men who have been beyond 300m remains very small; only an understanding of the basic mechanisms involved can ensure that such deep dives can continue in safety. □

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extremely strong, but much less certain is the connection between HSV type 2 and cervical cancer. R.P. Eglin and his colleagues (Institute of Virology, Glasgow) reported some very careful *in situ* hybridization experiments which showed low level expression of HSV-specific RNA sequences in pre-invasive and invasive carcinoma tissues, while two groups reported the induction of cervical carcinomas in animal model systems, W.B. Wentz and colleagues (Case Western Reserve University, Cleveland) using inactivated HSV type 2, and C. Minhui and associates (Hupei Medical College, Wuhan, China) using live virus. A very large prospective study to determine the risks associated with HSV type 2 infections in women is currently underway in Czechoslovakia (V. Vonka and colleagues, Institute of Sera and Vaccines, Prague) involving colposcopy, cytological smears and blood tests on 10,000 women selected at random.

One of the tantalizing questions about herpes viruses is how latent infections are maintained for long periods. What factors are important in the suppression of the infection? This is an area in which very little real progress has been made although it is generally accepted that specific antibodies are important in maintaining HSV latency. However some elegant experiments described by H. Openshaw (University of California, Sacramento) using a mouse model throw considerable doubt on this idea. Latent infections were established in mice which were protected during the primary infection by administration of immune antibody so that their own immune systems were not primed. It was shown in the majority of animals that as the passive antibodies faded the latent infections were maintained.

From the clinical point of view there are two areas which require urgent attention. The clinician would like to be in a position to offer some protection to a patient (e.g. a prospective organ recipient) who he knows will be at risk from these viruses in the immediate future. Such protection could be generated by a suitable vaccine. Secondly he needs effective drugs to control the more serious diseases associated with these viruses. There appear to be encouraging developments in both areas.

The problems associated with assessing the safety of attenuated herpesvirus vaccines are considerable since we have to take into account not only the primary infection but also recurrent disease and the oncogenic potential of these viruses. Attenuated VZV vaccine strains have been developed and are in use in some countries although the prevailing view, expressed by P.A. Brunnell (University of Texas), was that these vaccines should be used only in cases where patients are exposed to serious risk. An attenuated CMV vaccine has also

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*The meeting was held at the Institute of Marine Biomedical Research, Wilmington, N. Carolina and the proceedings will be published as *Techniques for diving deeper than 1500ft* by the Undersea Medical Society, Bethesda, Maryland.

(University of Chicago). Over a brief period there were 8 cases of HSV encephalitis at Massachusetts General Hospital, and since this is an extremely rare disease it was inevitably thought that the epidemic was caused by a particularly neurovirulent strain of virus. However, restriction enzyme analyses were made on the genome DNA of viruses isolated from all 8 patients and as the patterns were distinct from each other it was proven that the cases were caused by different virus strains.

The viruses may be particularly dangerous in two situations; infections of the newborn and of the immunosuppressed. CMV may cross the placenta and infect the fetus sometimes causing severe brain damage. HSV may be contracted from the infected genital tract of the

mother leaving the fetus with only a 50/50 chance of survival (A.J. Nahmias, Emory University, Atlanta). HSV, CMV and VZV are all common infections of immunosuppressed patients such as those under-going cancer therapy or organ transplantation. The diseases in these patients are often very severe and they are a significant cause of death. There were also suggestions that such diverse conditions as hardening of the arteries and psychotic depression (S. Sprecher, Institut Pasteur du Brabant) and even gastric ulcers (B.F. Vestergaard, Institute of Medical Microbiology, Copenhagen) might be initiated by recurrent HSV infections.

It has long been debated whether herpes viruses are involved in the induction of human cancers. Certainly the association between EBV and Burkitt's Lymphoma is

been developed and is undergoing clinical trials for renal transplant patients (S.C. Marker and H.H. Balfour, Jr., University of Minnesota). In the case of HSV and EBV where the oncogenic potentials are more clearly defined the development of attenuated strains has apparently been ruled out. Attention with HSV has been focused on nucleic acid-free subunit vaccines. The use of monoclonal antibodies in animal model systems has the potential to tell us which antigens are likely to elicit a protective response (R. Dix and colleagues, Veterans Administration Medical Center, San Francisco). These vaccines may also have problems associated with them since they are unlikely

to generate long term immunity and there may be dangers in delaying primary infection.

Chemotherapy of herpes virus infections has been hampered by the toxicity of the antiviral compounds available, so that treatment has been limited to topical application or desperate situations. However excitement is now running high as we appear to be entering a new era with the appearance of several low toxicity nucleoside analogues which are extremely effective against HSV. The most exciting of these compounds, Acyclovir, has generated very encouraging results in animals and the results of trials in humans are eagerly awaited. □

Free-electron lasers

from J.N. Elgin

In conventional laser systems, gain is obtained as a consequence of electronic transitions between bound states of the host substance. As a result, the gain band width of the laser is limited by the line width of the relevant bound-bound transition. In free-electron laser systems the electrons are free and gain is produced by the interaction of these free electrons, with the static magnetic field, as discussed below. Thus, these systems have the attractive advantage that their gain band width is, at least in principle, infinite.

Much interest has been generated in free-electron lasers since the recent experiments of Madey and coworkers at Stanford (*Phys. Rev. Lett.* 38, 717; 1976; *ibid* 38, 892; 1977). In their first experiment, the system was operated as a single pass amplifier and gain was demonstrated at 10.6μ , and in their second the system operated as a laser oscillator with output at 3.4μ .

For each case, the basic experimental arrangement can be represented schematically by the full outline in the figure. Here, a relativistic electron beam traverses a periodic magnetic structure

and, in so doing, emits synchrotron radiation into the forward direction. Stimulated emission of this radiation can occur in the vicinity of the resonance frequency (J.D. Lawson, *Nature* 277, 262; 1979)

$$\omega_r = c k_r = \frac{c\beta}{\beta k_0}$$

where $c\beta$ is the component of the electron velocity along the symmetry axis, and $k_0 = 2\pi/\lambda_0$ is the wave number of the static "wiggler" field. The gain curve for stimulated emission is markedly different from that encountered in conventional laser systems, being asymmetric about the resonance frequency ω_r (resembling somewhat an anomalous dispersion curve with resonance at ω_r). Only frequencies to one side of resonance are amplified, while those in the complementary region are strongly absorbed.

In each of the above experiments, electrons were passed only once through the apparatus, then discarded. In the experiment demonstrating gain at 10.6μ , it was found that about 0.2% of the electron's energy was converted to radiation without any evidence of saturation. It would

appear at first sight that this efficiency could be vastly improved simply by recirculating the electrons in a storage ring in which an r.f. cavity makes good all energy losses to radiation (i.e. in both the laser cavity and the ring), as indicated by the remaining part of the figure. In this way, efficiencies of, say, 20% or greater may be possible.

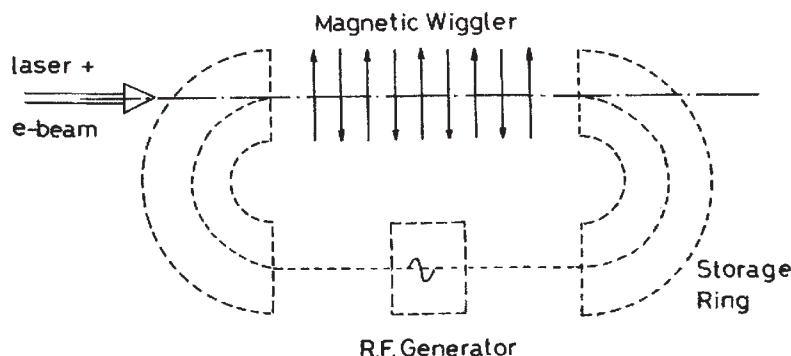
Unfortunately, energy spread of the input electron beam during transit through the laser cavity raises a nontrivial objection to the above scheme. Normally, the electrons enter the laser cavity with random optical phase so that $v_1 \cdot \epsilon$ — where v_1 is the transverse component of the electrons velocity and ϵ is the electric field strength of the laser radiation — is just as likely to be positive as it is negative. In other words, just as many electrons are extracting energy from the radiation field as are contributing energy to the field. The energy spectrum of the electrons therefore broadens (laser heating) and this effect is in general much larger than the (second-order) shift of the energy mean, which determines the net energy loss to the radiation field. Unless the recycling scheme can counteract this spread, it will become progressively larger with each transit, with a corresponding decrease in gain, until eventually the laser system ceases to function.

The simplest way in which the storage ring might be used to eliminate spread is quite simply to use radiation losses in the ring to damp out the spread. The radiative energy loss by a particle moving round the ring increases with increasing values of energy of the particle, and so a broad energy distribution necessarily narrows. The r.f. cavity makes good all radiative losses and the cycle is repeated, converging ultimately to the point where the spread has a constant value at input to the laser cavity. Of course, if this steady state distribution is too broad, the gain may become negligibly small and the laser will cease to function.

A more detailed study of the combined storage-ring laser system has recently been published by Deacon and Madey (*Phys. Rev. Lett.* 44, 449; 1980). The object here is to determine whether there are any possible conditions under which the electrons have isochronous orbits for transits through the complete system; that is, where the period for the complete orbit (laser plus storage ring) is independent of the initial conditions of the electron. Such a system necessarily has the required property of reversing the spread. The assumption is first made that the laser is operating in the Compton (or single particle) regime — which is the case for the experiments at Stanford — and the motion of the electrons through the laser cavity is accordingly modelled by the pendulum equation (Colson, *Phys. Lett.* 64A, 190; 1977)

$$\delta^2 \theta / \delta t^2 = -\Omega^2 \sin \theta$$

Here, $\theta = k_r \delta Z(t)$, where k_r is the wave number for the radiation field inside the



IN a recent paper in *Astrophysical Journal* (235, 625; 1980) H. Yoshimura has presented new results from computational simulations of non-linear astrophysical dynamos; in particular he has reproduced magnetic field reversals. This represents a contribution to the theory of field generation (i.e. dynamo action) in a sphere of electrically conducting fluid, the object of such investigations being to explain the observed features of the magnetic fields of the Earth and the Sun. In the terrestrial context, it is necessary to explain why the magnetic field of the Earth survives in a quasi-steady state over geological time scales much longer than the natural decay time of the field (of order 10^4 years). In the solar context, it is necessary to explain why the general magnetic field of the Sun evolves in an approximately periodic manner (with the same period as that of the sunspot cycle i.e. about 22 years) when the natural (ohmic) time scale for the Sun is of the order of 10^9 years.

Traditional dynamo theory starts from coupled equations for the velocity field $\mathbf{u}$ and the magnetic field $\mathbf{B}$ in the fluid region

$$\left. \begin{aligned} \frac{\delta \mathbf{B}}{\delta t} - \lambda \nabla^2 \mathbf{B} &= \mathbf{L} \{ \mathbf{u}, \mathbf{B} \} \\ \frac{\delta \mathbf{u}}{\delta t} - \nu \nabla^2 \mathbf{u} &= \mathbf{N} \{ \mathbf{u}, \mathbf{B} \} + \mathbf{F} \end{aligned} \right\} \quad (1)$$

In these equations $\mathbf{L}$ represents a differential operator that is linear in each of $\mathbf{u}$ and $\mathbf{B}$, $\mathbf{N}$ represents a nonlinear integral-differential operator, and $\mathbf{F}$ represents the force distribution, whatever this may be, which maintains the motion. Much controversy surrounds the nature of this force field in the terrestrial context, but in the solar

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Non-linear astrophysical dynamos

from H.K. Moffat

context it is certainly of thermal origin.

For a given force field, one would like to solve these equations, and demonstrate the existence of solutions for $\mathbf{B}$ which have the observed property in either context. Unfortunately, this task is far beyond either analytical solution, or the power of present computers. Approximate techniques must be adopted in order to reduce the problem to a level that will yield to the computer. A first dramatic stage has been achieved over the last 20 years, with the realisation that when the velocity field $\mathbf{u}$ is turbulent, the mean magnetic field $\langle \mathbf{B} \rangle$ satisfies a simpler equation

$$\left(\frac{\delta}{\delta t} - \lambda \nabla^2 \right) \langle \mathbf{B} \rangle = \langle \mathbf{L} \rangle \langle \mathbf{B} \rangle \quad (2)$$

where $\langle \mathbf{L} \rangle$ is an averaged differential operator which is in principle determinate in terms of the statistical properties of the turbulence. For different forms of this averaged operator, solutions of equation (2) are known of types that capture the main features either of the Earth's quasi-steady field, or of the Sun's quasi-periodic field.

The magnetic field itself influences the structure of the turbulence, through the second of Equations (1), and it is this "back reaction" effect that more recent theories are attempting to incorporate. First attempts in this direction were those of Stix (*Astron. Astrophys.* 20, 9; 1972) and Jepps (*J. Fluid Mech.* 67, 417; 1975), both of whom incorporated a fairly simple cut-off in the "regenerative" part of $\langle \mathbf{L} \rangle$ when the magnetic field exceeded

a level at which the back reaction effect was assumed to become significant. Yoshimura has developed this approach in a series of papers, in which he has considered increasingly sophisticated forms of the operator $\langle \mathbf{L} \rangle$, incorporating in particular a time-lag between the generation of a magnetic field and the (later) manifestation of its dynamic effects. This increasing sophistication in his models has enabled him to compute solutions of Equation (2) which resemble the observed fields of Earth and Sun in remarkable detail. As might perhaps be anticipated the more disposable parameters that are introduced in the assumed form of $\langle \mathbf{L} \rangle$, the more freedom there is at the end of the calculation to choose these parameters in a way that achieves agreement with observation. In particular, Yoshimura has now found solutions exhibiting reversals, like the well-documented occasional reversal of the Earth's field.

While Yoshimura's approach does not increase physical insight at the fundamental level, it does lead to the accumulation of an interesting catalogue of possible behaviours of solutions of equations of the form (2), when time delay is incorporated in the operator $\langle \mathbf{L} \rangle$. The approach however is open to criticism in that all the subtleties of the dynamical equation (1b) are parametrized in what many would regard as an unacceptably crude manner. Alternative approaches (e.g. that advocated by Malkus & Proctor (*J. Fluid Mech.* 67, 417; 1975) treat the dynamics in a less brutal manner, and although such approaches have not yet been pushed as far (in terms of results) as Yoshimura's, they hold more promise in the longer term for providing a convincing theory of dynamo action including proper recognition of the role of dynamic effects. □

cavity and δZ is the longitudinal deviation from resonance, and $\Omega^2 \alpha B_0 \epsilon$, the product of the wiggler field strength and the electric field amplitude of the generated radiation. The physics of electron bunches moving round storage rings is in general very complicated. However, as a first approximation, the ring system can also be modelled by a pendulum equation of the above type, where θ is now proportional to τ , the time displacement of a test particle from the nominal position, and $\Omega^2 \alpha V$, the amplitude of the applied r.f. field.

For either system (laser or ring) taken in isolation, the above equation predicts trapped particle solutions, where the particles oscillate backwards and forwards in the potential wells determined by Ω — a phenomenon well known in plasma physics. The length scale of the potential wells (or 'buckets') will of course be different in the two systems, being very much shorter in the laser cavity where they

are of the order of the optical wavelength. If the two systems are now coupled, as shown in the figure, we have a very complicated system indeed comprising as it does two coupled highly nonlinear differential equations. Clearly, such a system can only be properly studied using numerical techniques.

In their preliminary study of the problem, Deacon and Madey proceed by linearising the above system and, in so doing, are able to establish that isochronous orbits will occur provided the operating parameters of the system fall within defined limits. Within these limits, the large operating efficiencies (> 20%) referred to earlier should be realised. It will be interesting to see whether a more complete (numerical) study of the problem confirms these preliminary results.

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It is perhaps worth while mentioning two alternative schemes to the laser-ring system discussed above, both of which have received some theoretical study. In the first of these, an attempt is again made to reverse the energy spread of the electrons, but this time using echo techniques. Here, the effect is similar to one obtained in nonlinear optics where a randomising process can be reversed by a suitable coherent interaction of an optical pulse with the system. The second alternative uses a tapered wiggler magnet to slow down the electron bunches trapped in the optical potential wells referred to earlier, with the loss in energy of the bunches going into the radiation field. This latter method would of course be single pass only. Each possible method has its advantages and disadvantages and, at the time of writing, it is not yet clear which (if any) will provide the means of obtaining high efficiencies in free-electron laser systems. □

REVIEW ARTICLE

Biosynthesis of the pigments of life: formation of the macrocycle

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The organic nuclei of chlorophylls, haems, cytochromes and vitamin B₁₂ are biosynthesised from a single tetrapyrrolic intermediate which has an unexpected, rearranged structure. The mechanism of biosynthesis of this key intermediate has now been characterised in detail. Some of the information thereby obtained is also of use in the investigation of human diseases such as the porphyrias.

MANY vitally important functions in living systems are carried out by metal ions held as complexes within tetrapyrrolic macrocycles. Photosynthesis depends on chlorophylls (Mg²⁺ complexes), whilst the oxygen-carrying properties of haemoglobin and myoglobin are based on the Fe²⁺ complex (protohaem) of the macrocycle protoporphyrin-IX (compound 6). Electron transport, reduction of oxygen and hydroxylation reactions are mediated by a family of cytochromes which similarly depend on Fe²⁺ complexes (haems). Also, vitamin B₁₂ is a related complex of Co³⁺. Thus the entire group of macrocyclic pigments can fairly be called the pigments of life. This review outlines the highlights from 10 years of work aimed at discovering the biosynthetic pathways by which the organic macrocycles of these pigments are built. Only by having a full understanding of the normal biosynthetic processes can one pinpoint the failures which lead to disease.

Living systems tend to synthesise many complex molecules by assembling and/or modifying a relatively small number of starting materials¹. This is certainly true for the macrocyclic pigments. Indeed, all the natural pigments mentioned above are biosynthetically derived from a single intermediate, uroporphyrinogen-III (3) (uro'gen-III). In plants, animals and most bacteria, the major forward pathway then involves decarboxylation of uro'gen-III (3) to yield coproporphyrinogen-III (copro'gen-III) (4), which by oxidative decarboxylation produces protoporphyrinogen-IX (proto'gen-IX) (5). Aromatisation of proto'gen-IX (5) gives protoporphyrin-IX (6) from which one set of pigments is built, as shown in Fig. 1. A second branch from uro'gen-III (3) leads via cobyrinic acid (7) to vitamin B₁₂; here, the key modifying process is not oxidation but methylation on carbon.

This central position for uro'gen-III (3) in a family of pigments of immense biological importance, emphasises the value of understanding its biosynthesis.

Building blocks and enzymes

Shemin, Granick, Bogorad, Neuberger and Rimmington had shown in pioneering work² that two enzymes are required to catalyse the conversion of four molecules of the monopyrrole porphobilinogen (PBG) (1) into uro'gen-III (3) and ammonia (Fig. 1). The names of these enzymes 'PBG-deaminase' and 'uro'gen-III cosynthetase' will be shortened here to deaminase and cosynthetase. In the absence of cosynthetase, deaminase catalyses the conversion of PBG into uro'gen-I (2), an unnatural isomer but the one expected from simple head-to-tail combina-

tion of four PBG units. However, uro'gen-I (2) is not converted into uro'gen-III (3) by cosynthetase nor by the complete deaminase-cosynthetase system. It is fascinating that living systems have developed during evolution a highly specific method of catalysing a rearrangement process which produces the unexpected type-III isomer (3), having adjacent propionate groups on rings C and D. How do the two enzymes work together to achieve the synthesis and what are the intermediates? These problems have a special fascination because four identical building blocks (PBG) are used for uro'gen-III (3); because they are identical new approaches had to be devised to solve this riddle.

Nature of the rearrangement

To produce uro'gen-III (3) from PBG (1), deaminase-cosynthetase must be capable at some stage of breaking the bond linking the C-11 methylene group to its pyrrole nucleus for one or more of the four PBG units. Obviously, there are many ways in which four pyrrole rings and the four methylene groups can be shuffled to produce uro'gen-III (3). It is a measure of the intense interest in this problem that at least 25 hypothetical schemes have been proposed for the biosynthetic process.

The various possible ways of building uro'gen-III (3) from PBG (1) differ in which of the four bridge carbons (C-5, 10, 15 and 20) are still attached to the same carbons at the same pyrrole nuclei as they were in the original PBG units. Methods had therefore to be found to study processes which break established C—C bonds and make new ones. ¹³C-NMR proved to be a suitable method. The principle of this approach is simple; the ¹³C-NMR signal from a ¹³C-atom is affected by another ¹³C-atom only if both are in the same molecule. Parallel synthetic work showed^{3,4} that two directly bonded ¹³C-atoms (●—●, Fig. 1, structure (6)) give characteristic ¹³C-NMR signals, easily distinguishable from those given by ¹³C-atoms separated by three bonds (for example, ▲—C—N—▲, structure (6)). These in turn were clearly different from the signals given by two ¹³C-atoms in different molecules.

PBG (1a, Fig. 2) was synthesised carrying 90 atom% ¹³C at both C-2 and C-11; thus 81% of the PBG molecules carried two ¹³C-atoms. This product (one part) was diluted with unenriched PBG (four parts) in which a negligible proportion of molecules carry two ¹³C-atoms at C-2 and C-11. Incubation of the resultant PBG sample with an enzyme system rich in deaminase-cosynthetase from *Euglena gracilis* first produced uro'gen-III (3a). However, because the biological system contained all the



^{13}C -NMR then proved^{5,6} that the ^{13}C -pattern of the labelled protoporphyrin-IX was as shown in structure (6). The geometrical shapes each represent a ^{13}C -atom and those giving the same shape were proved to be present in the same molecule. As protoporphyrin-IX is formed from uro'gen-III without disruption of the macrocycle (Fig. 1) the results for the former also hold good for the latter (3a, Fig. 2). Exactly the same pattern was found^{5,6} when the experiment was repeated: (i) with an enzyme preparation from chicken's blood; and (ii) using *Propionibacterium shermanii*. It follows that the deaminase-cosynthetase system assembles four PBG molecules to form uro'gen-III in exactly the same manner in a photosynthetic alga, in avian blood and in a bacterium, and the mode of assembly, not considering the mechanism at this stage, is that illustrated in Fig. 2.

Thus three PBG units are built intact into uro'gen-III (3) together with one intramolecularly rearranged unit corresponding to ring D and C-15. In principle, the single rearrangement could occur at any stage in the overall process, at the monopyrrole, dipyrrole, tripyrrole or tetrapyrrole levels. In fact, many experiments with mono- and dipyrroles pointed strongly to rearrangement at the tetrapyrrole level⁷⁻⁹, in contrast to other claims¹⁰⁻¹².

To determine what happens non-enzymatically to the bilane samples (9b and 9c), they were allowed to ring-close at pH 7, and the product in each case was shown to be almost pure uro'gen-I (2b and 2c), with only traces of other isomers^{13,14}. Thus no significant chemical rearrangement occurred and the foundation was established for the key enzymatic experiments. These involved separate incubation of each of these diluted samples (9b and 9c) with deaminase-cosynthetase. Strikingly, the product in each case now contained 80% uro'gen-III (3b and 3c) and only 20% of uro'gen-I (2b and 2c). The same bilane in the unlabelled form was prepared by a different method¹⁵ and deaminase-cosynthetase from *P. shermanii* partly transformed it into uro'gen-III (type-III: type-I ratio 16:84). ¹³C-NMR of the type-III isomer isolated from one experiment showed that the two ¹³C-atoms, shown as ● in Fig. 3, 3b, had become bonded intramolecularly. The same was true in the other experiment for the two ¹³C-atoms marked ▲ (Fig. 3, 3c). The ¹³C-spectra were run on the derived coproporphyrin-III tetramethyl ester (10b and 10c), obtained by aromatisation of uro'gen-III with iodine, decarboxylation of the acetic acid side chains and esterification. The ▲ labels in the second experiment above lie at the two ends of the bilane (9b) so the further important conclusion could be drawn that the entire bilane (9c) was converted intact into uro'gen-III (3c).

The above studies depended on quantitative determinations of the amounts of uro'-gen-III (3) and uro'-gen-I (2) (and also the type-II and type-IV isomers) present in a mixture of these macrocycles. In carefully selected conditions, separation of the four coproporphyrin methyl esters derived from the uro'-gens was achieved in a single HPLC run, a method which could be adapted to the analysis of porphyrin isomers from porphyric patients.

It is now firmly established that the deaminase-cosynthetase system brings about head-to-tail assembly of four PBG units to form an unrearranged bilane (8). A group X is shown in Fig. 3 because it was clear that the $^+\text{NH}_3$ -group of PBG (1) (and similarly the $^+\text{NH}_3$ -group of the $^+\text{NH}_3\text{CH}_2$ -bilane (9a)) might be enzymatically replaced before the final ring-closure with rearrangement^{14,16}. It was envisaged that the group X could be some nucleophilic group on the enzyme or an external nucleophile. The unrearranged bilane (8) is then enzymatically transformed intact into uro'gen-III (3) with intramolecular inversion solely of ring D, exactly as found earlier for PBG (1). Rings A, B, C and D of the bilane (8) correspond respectively to rings A, B, C and D of uro'gen-III.

Is there an obligatory order of assembly of the bilane (8)? If so, does the first PBG unit enzymatically bound eventually become ring A of bilane (8), and so ring A of uro'gen-III, followed by sequential addition of ring B, ring C and finally ring D, or vice versa?

(3a) Urogen - III

 Mode of assembly

Fig. 2

thetase was treated with a deficiency ($\sim 0.5 \mu\text{mol}$) of unlabelled PBG (1). This was designed to produce the greatest 'loading' on to the enzymatic site to which the first PBG binds. The corresponding 'loadings' at the other sites should decrease in a stepwise way so that the last site to be filled should carry least. The loading referred to covers pyrrolic material on that site whether it be monopyrrole (for example Fig. 4, 12) or part of a di- (for example, 13), tri- (14) or tetrapyrrole (8). An excess of 90 atom% $[11\text{-}^{13}\text{C}]\text{PBG}$ (Fig. 2, 1b) was then added to chase through the bound pyrrolic species to form uro'gen-III (3) which as earlier was converted into coproporphyrin-III methyl ester (11) for NMR study.

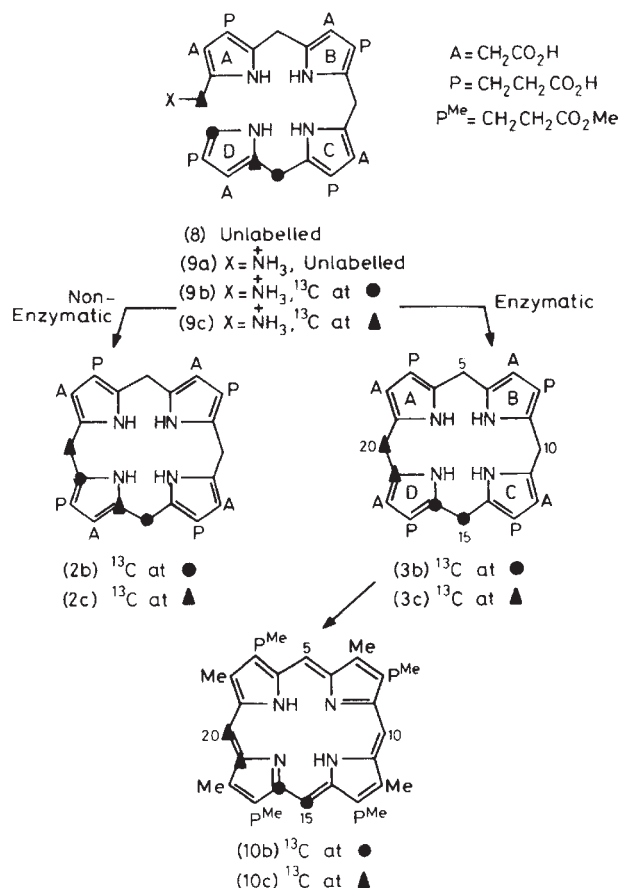


Fig. 3

The ^1H -NMR signal from a ^1H -atom attached to, for example, C-20 of compound (11) will give a singlet if C-20 is a ^{12}C -atom but a widely split doublet will appear if C-20 is a ^{13}C -atom. Thus the size of the central ^1H -singlet is a measure of the ^{12}C content at that 'bridge' position. It can now be seen clearly from Fig. 5 that the ^{12}C -content is greatest at C-20 and that the ^{12}C -levels at the other bridge carbons fall in the order $\text{C-5} > \text{C-10} > \text{C-15}$. Thus¹⁷ the first PBG unit to bind becomes ring A (with C-20), the second ring B (with C-5), the third ring C (with C-10) and the last ring D (and C-15). The same conclusion is reached independently in a recent study based on ^{14}C -labelling¹⁸.

The intermediate hydroxymethylbilane

The studies so far made use of the complete deaminase-cosynthetase system but further progress demanded the individual enzymes; they had not been separated previously from *Euglena gracilis*. Classical work^{19,20} showed that cosynthetase from other sources is thermally less stable than deaminase, and this holds true for the *Euglena* enzymes. However, both enzymes from *Euglena* are sufficiently stable to be cleanly separated chroma-

tographically and handled with workable levels of activity (ref. 21 and G.W.J.M., E.McD. and A.R.B., in preparation).

When deaminase-cosynthetase acted on the $^+\text{NH}_3\text{CH}_2$ -bilane (9a), as outlined above, there was enzymatic acceleration of the ring-closure, relative to the non-enzymatic rate, and the formation of uro'gen-III (3) showed no lag phase. However, when the $^+\text{NH}_3\text{CH}_2$ -bilane (9a) was incubated with deaminase (free of cosynthetase), there was an initial lag before acceleration of the formation of uro'gen-I (2). An even more striking lag was observed²² in the production of uro'gen-I (2) by deaminase from PBG (see Fig. 6); there was no lag in formation of uro'gen-III (3) from PBG when the deaminase-cosynthetase system was used.

Clearly, an intermediate substance is released into the medium when deaminase alone acts on PBG (1) or on the $^+\text{NH}_3\text{CH}_2$ -bilane (9a); proof will be given later that the same intermediate is formed from both. What is its structure?

The intermediate was first generated from PBG (1) using deaminase to point A in Fig. 6 and it was then allowed to ring-close. The product was more than 99% uro'gen-I (2), showing that four PBG units had been joined head-to-tail without rearrangement. The intermediate was then generated as before but from $[11\text{-}^{13}\text{C}]\text{PBG}$ (Fig. 2, 1b) and, after the enzymatic reaction was quenched, ^{13}C -NMR of the intermediate showed it to contain one $\text{HO}^{13}\text{CH}_2$ -pyrrole residue and three methylene groups in the environment pyrrole- $^{13}\text{CH}_2$ -pyrrole. These results proved²² that the intermediate produced from PBG (1) by deaminase alone is the unrearranged hydroxymethylbilane (Fig. 4, 15), abbreviated below to HOCH_2 -bilane. Similar ^{13}C -NMR studies showed¹⁷ that deaminase converts the $^+\text{NH}_3\text{CH}_2$ -bilane (9a) into this same HOCH_2 -bilane (15).

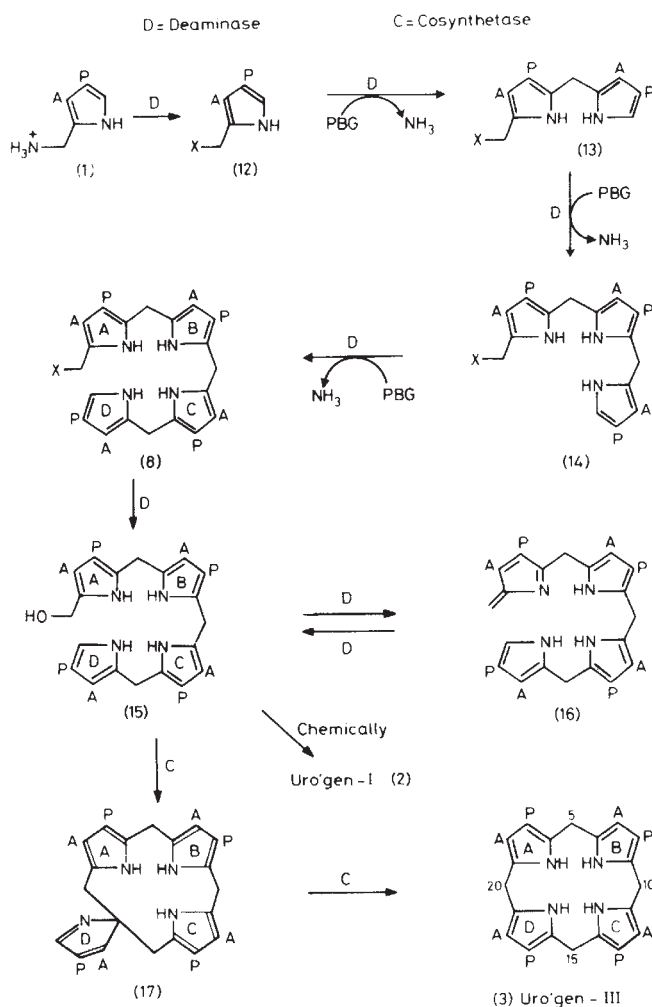


Fig. 4

We can look back at the comments made earlier concerning the group X in Fig. 3; it is now clear that when deaminase acts alone on PBG, the final nucleophile is water and X = OH.

Rigorous confirmation of the HOCH₂-bilane structure (15) for the intermediate came from its synthesis. This presented more than the usual challenge since the half life of the HOCH₂-bilane in physiological conditions is only a few minutes. Synthesis was achieved²³ by generating the labile HOCH₂-pyrrole residue only at the penultimate stage. This synthetic HOCH₂-bilane (15) was chemically and spectroscopically identical with the natural intermediate. The stage was thus set for enzymatic comparisons of the natural and synthetic HOCH₂-bilane (15).

The roles of deaminase and cosynthetase

Deaminase. The HOCH₂-bilane (15) was shown to be the endproduct of deaminase action on PBG by generating the bilane as earlier to point A, Fig. 6, and allowing it to ring-close chemically. A second equivalent portion of (15) was treated at point B, Fig. 6, with additional deaminase. The two rates, from A and B, of uro'gen-I (2) formation were essentially the same. So deaminase is not an enzyme for ring-closure and, in the absence of cosynthetase, its product is the unrearranged HOCH₂-bilane (15)²².

This product does not react chemically at an appreciable rate with added nitrogenous nucleophiles such as ⁺NH₄ or NH₂OH, but when the HOCH₂-bilane (15) is treated with these nucleophiles in the presence of deaminase there is essentially complete conversion into ⁺NH₃CH₂-bilane (9a) and HONHCH₂-bilane

(8, X = HONH), respectively. The nucleophiles are thus trapping a substance, probably enzyme-bound, which is more reactive than the HOCH₂-bilane (15) and can be derived enzymatically from it²². The properties of this species suggest that it is the enzyme-stabilised methylenepyrroline (16). The earlier important observations of such nucleophilic trapping^{24,25} fall into place on this view.

These trapping experiments have valuable mechanistic implications but the major point is this: with the unrearranged tetrapyrrole system (15) having been built, deaminase has done its job ready for cosynthetase to play its part.

Cosynthetase. The HOCH₂-bilane (15) generated enzymatically to point C, Fig. 6, was treated initially with deaminase-cosynthetase and the result was dramatic²². Virtually instantaneous ring-closure occurred with rearrangement to produce uro'gen-III (3), Fig. 6.

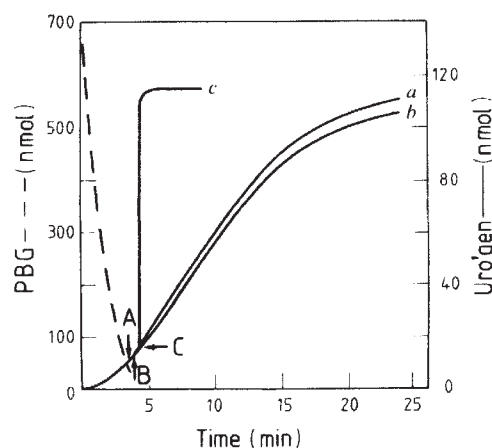


Fig. 6 Formation of hydroxymethylbilane (15) from PBG by deaminase followed by ring-closure from points A, B and C: a, without additional deaminase; b, with additional deaminase; c, with added deaminase-cosynthetase.

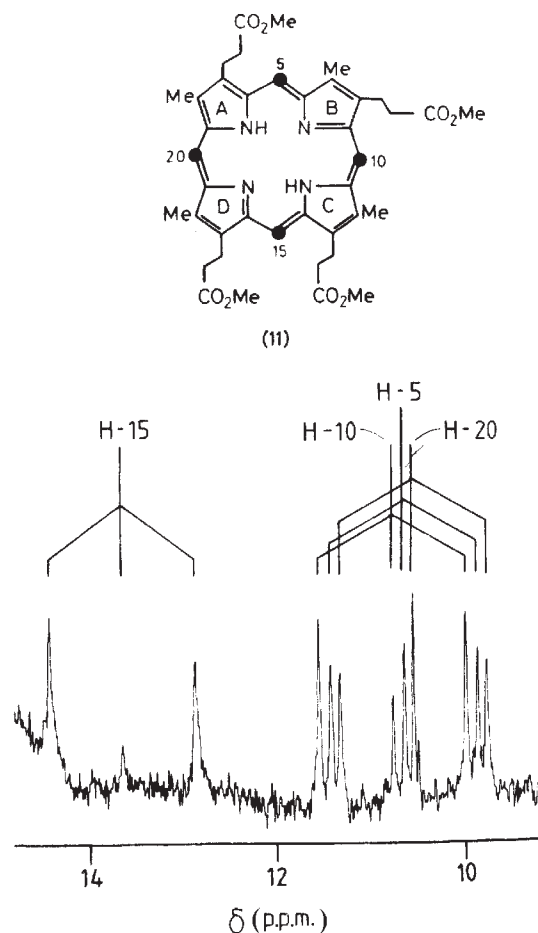


Fig. 5 ¹H-NMR signals from positions 5, 10, 15 and 20 of coproporphyrin-III tetramethyl ester (11) from experiment on order of assembly of the pyrrole rings; the relative positions of the signals, spread by added shift reagent, had previously been assigned¹⁴.

It was satisfying to find that cosynthetase alone acted in the same very rapid way on the HOCH₂-bilane (15) to form >98% isomerically pure uro'gen-III (3); the same result was obtained whether the HOCH₂-bilane (15) had been enzymatically generated or chemically synthesised²³. Table 1 gives a comparison of the two preparations and particular attention is drawn to the quantitative agreement of the kinetic data. Cosynthetase is thus the ring-closing and rearranging enzyme; its substrate is now known and is available synthetically.

Assay for cosynthetase. The foregoing results are of relevance to medical studies of certain porphyrias. In the past, no direct assay was available for cosynthetase; its presence or absence in an enzyme preparation could be crudely checked by combining it with deaminase and determining the proportion of uro'gen-III (3) in the macrocyclic products formed from PBG. The availability of synthetic HOCH₂-bilane (15) allows rapid, direct and quantitative assay for cosynthetase and so should allow identification of porphyria patients deficient in this enzyme.

Deaminase-cosynthetase. Bearing in mind knowledge summarised so far, there are two ways in which deaminase and cosynthetase could operate in forming uro'gen-III (3). (i) They could be completely independent enzymes such that in their natural state the HOCH₂-bilane (15) from deaminase is released into the medium to be picked up by cosynthetase; the two enzymes can certainly function quite independently *in vitro*. (ii) Deaminase and cosynthetase, each with its independent active site, may be closely associated by physical binding and uro'gen-III could be formed by transfer of the HOCH₂-bilane within the complex. The experimental distinction between (i) and (ii) is important and requires more work. It is known^{21,26} that

deaminase and cosynthetase can bind to each other but it remains to be seen whether evolutionary pressures have selected what would be expected to be the more efficient possibility (ii); we hope that time, on our human scale, will allow the distinction to become evident.

Valuable enzymatic work in this area has been reported independently by Scott's group²⁷ but their chemistry and conclusions are completely different from ours; evidence from many angles²³ does not favour their view.

Table 1 Comparison of synthetic and natural HOCH₂-bilane (15)

	<i>t</i> _i (pH 8.25 at 37 °C)	% Uro'gen-III formed by cosynthetase alone	<i>V</i> _{max} for cosynthetase*
Synthetic	4.0 min	>98	151
Natural	4.2 min	98	148

* μmol uro'gen produced at pH 8.25 at 25 °C per hour, per ml of cosynthetase preparation.

Present knowledge and prospects

The biosynthetic pathway which has emerged from the work outlined here is illustrated in Fig. 4. Four units of PBG (1) are assembled head-to-tail by deaminase, starting with ring A and building round to ring D to form the unrearranged bilane (8). In the absence of cosynthetase, the bilane is released into the medium as hydroxymethylbilane (15). Deaminase has then done its job; it is not an enzyme for ring-closure, although it does equilibrate the HOCH₂-bilane (15) with a more reactive species (probably enzyme stabilised) which has properties expected of the methylenepyrroline (16).

Cosynthetase then takes charge by ring-closing the HOCH₂-bilane (15) at high speed to form uro'gen-III (3). This involves an intramolecular rearrangement which affects only ring D and the atoms which become C-15 and C-20 of uro'gen-III (3) (see Fig. 3 for what joins to what). The most probable intermediate in this rearrangement is the spiro-system (17).

We are now seeking direct evidence for the intermediate(s) between the HOCH₂-bilane (15) and uro'gen-III (3) and also information about the nature of group X (Fig. 4). The recently acquired knowledge reviewed here opens the way to fascinating opportunities in the medical and enzymatic areas where exciting advances should be possible.

We thank many colleagues, whose names appear in the literature, and the Nuffield Foundation, the SRC and Roche Products for financial support.

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ARTICLE

Inverted terminal repetition in vaccinia virus DNA encodes early mRNAs

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Vaccinia virus DNA contains a long inverted terminal repetition of MW ~6.8 × 10⁶. A fragment of MW 6.3 × 10⁶ from this repetition has been cloned in coliphage λ and used to isolate RNA from virus-infected cells. Electron microscopy indicates that early RNAs are transcribed from the repeated sequence and cell-free translation shows that the RNAs code for polypeptides.

IDENTICAL sequences are present at the two ends of the genome of many viruses, presumably to fulfill an essential function in DNA replication^{1–7}. However, the long (~7 × 10⁶ dalton) inverted terminal repetition in vaccinia virus^{6,7} appears

to be transcribed⁷, suggesting that the region may also encode viral proteins. To investigate this interesting possibility, we have used coliphage λ to clone a fragment of molecular weight (MW) 6.3 × 10⁶ from the end of the 122 × 10⁶ MW vaccinia virus

genome and have used the recombinant DNA to isolate RNA from virus-infected cells. Electron microscopy indicates that early RNAs are transcribed from the repeated sequence, and cell-free translation shows that these RNAs code for polypeptides.

Length of the terminal repetition

Restriction endonuclease analyses were undertaken both to confirm the length of the inverted terminal repetition, which was estimated by electron microscopy to be of MW 6.8×10^6 daltons for the WR strain of vaccinia virus⁷, and to select suitable DNA fragments for cloning. *Hind*III digestion of vaccinia virus DNA produces two end fragments of MW approximately 17.8 and 13.4×10^6 (ref. 7). When these were further digested with *Hinc*II and analysed by agarose gel electrophoresis, 8 of the 15 bands from the larger (*Hind*III B) end fragment co-migrated with 8 of the 14 bands from the smaller (*Hind*III C) end fragment (Table 1). Similarly, 8 common bands were detected by *Hpa*II digestion of the two *Hind*III end fragments (Table 1). The sums of the common *Hinc*II and *Hpa*II fragments were 6.6×10^6 and 6.2×10^6 daltons, respectively, which is consistent with their deriva-

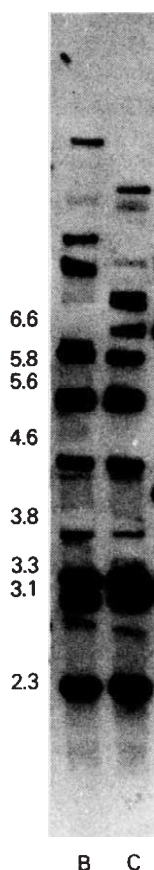


Fig. 1 Autoradiograph showing agarose gel electrophoresis of end fragments obtained by partial *Hinc*II digestion of *Hind*III fragments B and C. Approximately 1 μ g of purified *Hind*III fragments B and C were digested separately with 1 unit of *Hinc*II. At 1 to 30 min after addition of enzyme, portions of the incubation mixture were removed and digestion was stopped by addition of EDTA. Samples were combined and electrophoresed on a 0.8% agarose gel. DNA fragments were transferred to nitrocellulose⁸ and probed with the *Sal*I end-fragment (MW 2.35×10^6) labelled with 32 P by nick-translation¹⁴. Molecular weights of the partial digestion products were determined by comparison of their electrophoretic mobilities with those of λ and adenovirus DNA fragments of known size. B, Partial products from *Hind*III fragment B; C, partial products from *Hind*III fragment C.

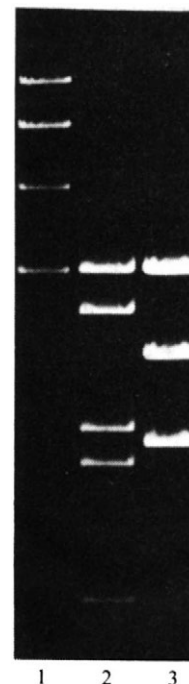


Fig. 2 Ethidium bromide-stained agarose gel showing fragment patterns produced by cleavage of the recombinant phage DNA (track 1) and by cleavage of vaccinia virus DNA *Hind*III fragments B (track 2) and C (track 3) with *Eco*RI. A mixture of the two *Hind*III end fragments B and C was isolated from sucrose gradients⁷ and the cross-links were removed by treatment with a single-strand specific DNase isolated from vaccinia virions as described previously^{7,9}. One μ g of octameric *Eco*RI linker (Collaborative Research) was phosphorylated with polynucleotide kinase and ligated to approximately 5 μ g of *Hind*III fragments as described¹⁵ except that ligation was performed at 11.5°C for 17.5 h. After ligation, linker oligomers were removed by chromatography on a Sepharose 6B column. The DNA was recovered in the void volume, extracted with phenol and precipitated with ethanol. The DNA was then cleaved with *Eco*RI and the fragments were ligated to the arms of λ gt WES- λ B (ref. 16) which had been purified by chromatography on RPC5. Recombinant DNA molecules were encapsidated by 'in vitro packaging'¹⁷ and the resulting phage were used to infect *E. coli* K12 DP50/supF (ref. 16) under P2-EK2 conditions in line with NIH guidelines for recombinant DNA experiments. Phage containing the *Eco*RI end-fragment were detected by the plaque-hybridisation technique¹⁸ using 32 P-labelled *Sal*I end-fragment (MW 2.35×10^6) of vaccinia virus DNA as a probe. Positive phage plaques were picked and used to infect a 5 ml culture of DP50/supF cells. The phage were partially purified¹⁹, after which the DNA was extracted, cleaved with *Eco*RI, and analysed on a 15 cm long 0.8% agarose gel. *Eco*RI digests of *Hind*III end fragments B and C, which were isolated from 0.6% agarose gels and purified by binding to glass-powder²⁰, were analysed on the same gel. The smallest *Eco*RI fragments of *Hind*III fragment B (MW $\sim 0.52 \times 10^6$) and *Hind*III fragment C (MW $\sim 0.7 \times 10^6$) have both run off the gel.

tion from a 6.8×10^6 dalton terminal repetition. This was confirmed by showing that the sizes of 8 partial *Hinc*II digestion products derived from the two ends of the genome were identical. After partial *Hinc*II digestion of *Hind*III fragments B and C and agarose gel electrophoresis, the terminal 2.35×10^6 dalton *Hinc*II fragment and all partial digestion products containing this sequence were identified by hybridisation to a 32 P-labelled 2.35×10^6 dalton end fragment probe. Figure 1 reveals that the first eight *Hinc*II sites occur in the same locations in *Hind*III fragments B and C, thereby generating eight common bands. From this point on, the partial digestion products from *Hind*III fragments B and C diverged (Fig. 1). The largest common partial end fragment is 6.6×10^6 daltons in agreement with the sum of the eight co-migrating *Hinc*II fragments (Table 1). The partial digestion method was also used to show eight

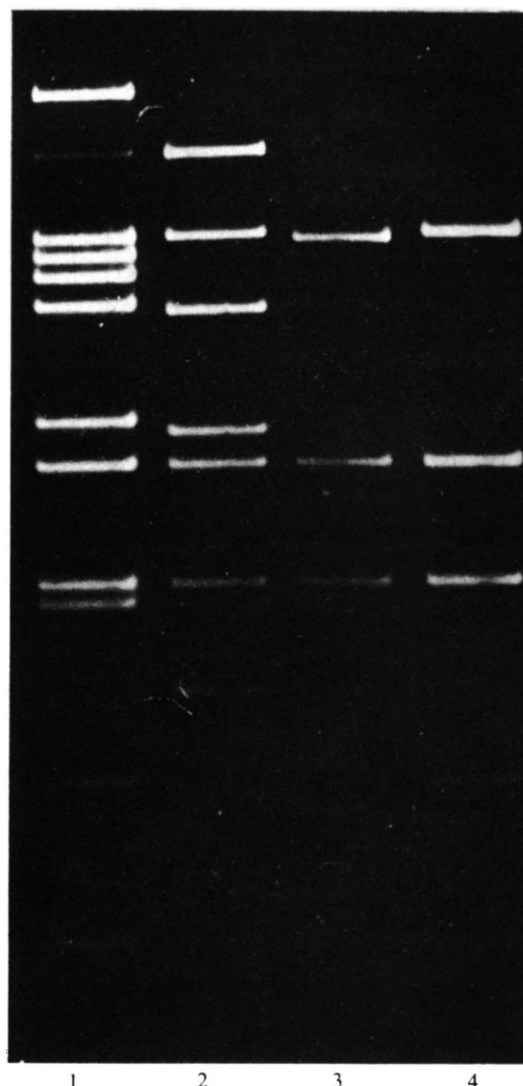


Fig. 3 Ethidium bromide-stained agarose gel showing the fragment patterns produced by cleavage of the cloned DNA insert and of authentic vaccinia virus DNA with *HpaII*. *HindIII* fragments B (track 1) and C (track 2) were isolated from 0.6% agarose gels by binding to glass powder²⁰ and cleaved with *HpaII*. Recombinant phage DNA was cleaved with *EcoRI* and the 'insert' was purified and cleaved with *HpaII* (track 3). Authentic *EcoRI* end fragment was obtained by cleavage of isolated *HindIII* fragment C from vaccinia virus DNA with *EcoRI* and isolation of the *EcoRI* end fragment. The purified fragment was further digested with *HpaII* (lane 4). Electrophoresis was carried out in 1.4% agarose gels.

identical *HpaII* sites at both ends of the vaccinia virus genome (not shown). Thus restriction endonuclease analyses indicated that the inverted terminal repetition is greater than 6.6×10^6 daltons.

Cloning the terminal repetition

HincII and *HpaII* were useful for determining the minimum length of the terminal repetition because they make a large number of cuts in that region. However, to obtain a fragment for cloning, we required a restriction endonuclease that made its first cut just before the junction of the repeated and unique sequences. Screening for such an enzyme was simplified by an unusual feature of vaccinia virus DNA. Because the two strands of DNA are covalently linked at their ends⁹, terminal fragments produced by restriction endonucleases can be identified by their ability to renature rapidly¹⁰. As anticipated, agarose gel electrophoresis showed a single rapidly renaturing end fragment when the first cut occurred within the terminal repetition whereas two end fragments were found when the first cut was beyond the 6.8×10^6 dalton repetition. From this survey, we noted that *EcoRI* produced a single 6.3×10^6 dalton rapidly renaturing end fragment from whole vaccinia virus DNA and the same size fragment on sub-digestion of either of the two large *HindIII* end fragments (Table 1; tracks 2 and 3, Fig. 2). Accordingly, the *EcoRI* end fragment was selected for cloning.

The strategy used to insert the *EcoRI* end fragment of vaccinia virus DNA into coliphage λ , detailed in Fig. 2 legend, involved cleavage of the covalently linked termini of isolated vaccinia *HindIII* end fragments with a single-strand specific DNase;

Table 1 Molecular weights $\times 10^{-6}$ of *HincII*, *HpaII* and *EcoRI* restriction fragments produced by subdigestion of *HindIII* fragments B and C

Restriction endonuclease <i>HindIII</i> fragment	<i>HincII</i>		<i>HpaII</i>		<i>EcoRI</i>	
	B	C	B	C	B	C
	2.80		3.80		6.30*	6.30*
	2.43			3.00	5.15	
	2.35*	2.35*	2.30*	2.30*		4.41
	2.24		2.15		3.30	
		2.20	2.05			3.10
	1.48		1.92		2.86	
		1.32		1.90	1.70	
	1.28		1.50			0.70
	1.00			1.45	0.57	
	0.97	0.97†	1.40	1.40		
	0.93	0.93	1.10	1.10		
	0.85	0.85†	0.98			
		0.73		0.85		
		0.68	0.66	0.66		
	0.63	0.63		0.55		
		0.58	0.43			
	0.47†	0.47	0.40			
		0.25†		0.38		
	0.20	0.20	0.25	0.25		
	0.17	0.17	0.20	0.20		
	0.15		0.17	0.17		
			0.15	0.15		
Sum of total	18.4	14.4	19.5	14.4	19.9	14.5
Sum of common		6.6		6.2		6.3

* Fragments identified by agarose gel electrophoresis after rapid renaturation as containing cross-links.

† Intense ethidium bromide-staining bands suggesting two fragments of similar molecular weight.

blunt-end ligation of an octameric linker containing the *EcoRI* site to the terminus; *EcoRI* digestion of the product and ligation of the vaccinia DNA fragment to λ 'arms'. After *in vitro* packaging of the recombinant DNA, *Escherichia coli* were infected and plaques were identified by hybridisation using the 2.35×10^6 dalton ^{32}P -labelled *SalI* end fragment of vaccinia virus DNA as a probe. For further identification, the recombinant phage DNA was purified and digested with *EcoRI*. Four

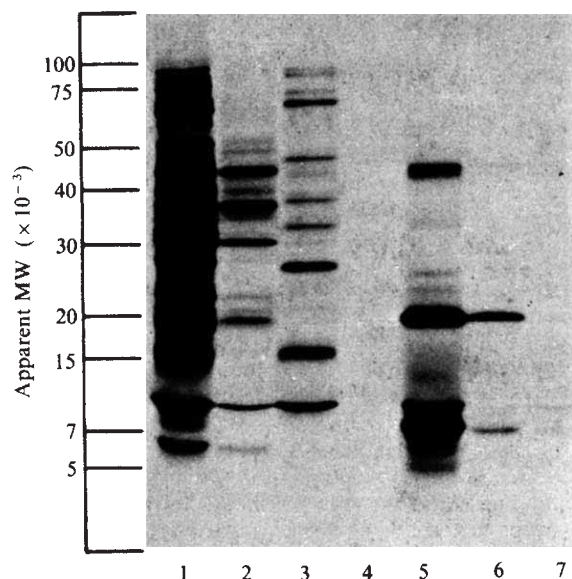


Fig. 4 Autoradiograph of a polyacrylamide gel showing the polypeptides synthesised in a reticulocyte cell-free system programmed with RNA selected by hybridisation to the cloned terminal repetition of vaccinia virus DNA. Cytoplasmic RNA was purified from HeLa cells which had been infected with approximately 30 plaque-forming units of purified vaccinia virus per cell¹¹. Immediate early, early and late RNA were prepared from cells infected for 4 h in the presence of $100 \mu\text{g ml}^{-1}$ cycloheximide, $40 \mu\text{g ml}^{-1}$ cytosine arabinoside or for 6 h without inhibitors, respectively. Approximately $210 \mu\text{g}$ of each RNA preparation was hybridised to 0.2 pmol of recombinant DNA immobilised on nitrocellulose filters in $300 \mu\text{l}$ of 80% formamide, 0.4 M NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA and 0.1% SDS at 37°C in a shaking water bath for 18 h. At the end of the hybridisation, the filters were stringently washed to remove unhybridised RNA²¹. This consisted of three washes at room temperature with 4 ml of 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 0.1% SDS, then three washes with 10 mM Tris-HCl pH 7.5, 1 mM EDTA. The filters were then washed twice using 0.4 ml of hybridisation solution lacking SDS at 37°C for 15 min and finally in 0.4 ml of 80% formamide, 40 mM PIPES pH 6.4, 1 mM EDTA at 37°C for 15 min. Hybridised RNA was eluted in 0.2 ml of water at 100°C for 2 min, and then alcohol precipitated with $20 \mu\text{g}$ of calf liver tRNA (Boehringer Mannheim). The precipitate was washed twice with 70% ethanol and dried *in vacuo*. This RNA was used to programme cell-free protein synthesis in $10\text{-}\mu\text{l}$ reactions containing message-dependent reticulocyte lysate^{12,22}. Samples of each reaction ($2 \mu\text{l}$) were prepared for electrophoresis and analysed on a 9 cm long, SDS polyacrylamide (20% T, 0.33% C) gel^{11,12}. An 8-h fluorographic exposure is shown. The reticulocyte lysate was programmed with tRNA only (track 4), with tRNA and $1 \mu\text{g}$ of unpurified immediate early, early or late RNA (tracks 1, 2 and 3, respectively), or with the immediate early, early or late RNA purified by hybridisation to recombinant DNA sequences (tracks 5, 6 and 7, respectively). The apparent space at $\sim 15,000$ daltons, and the compression just below it, are due to large amounts of unlabelled globin from the reticulocyte lysate. Similar polypeptides were detected when RNA, purified by solution hybridisation and isolation of the hybridised RNA on potassium iodide density gradients²³, was translated.

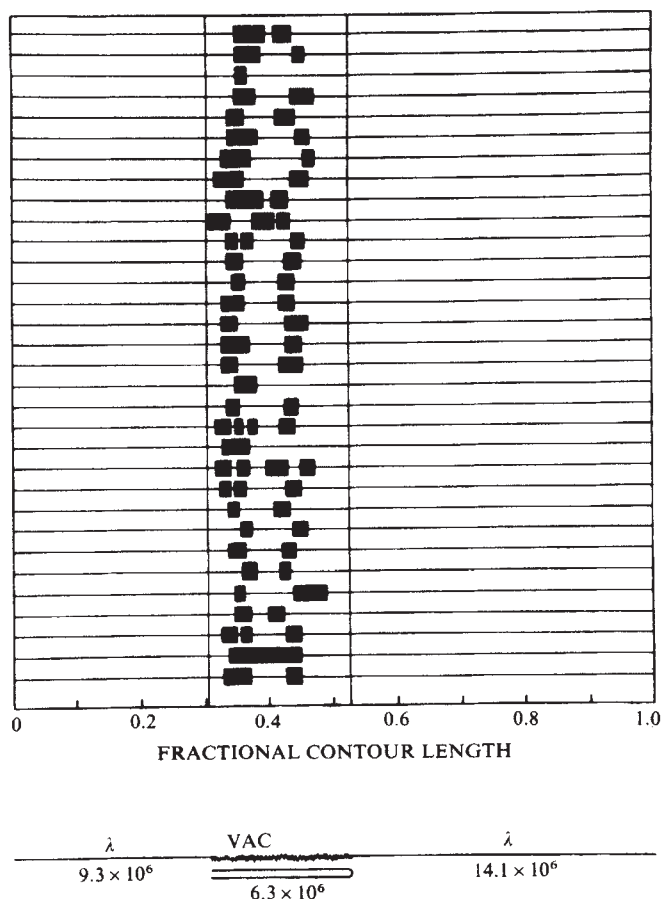
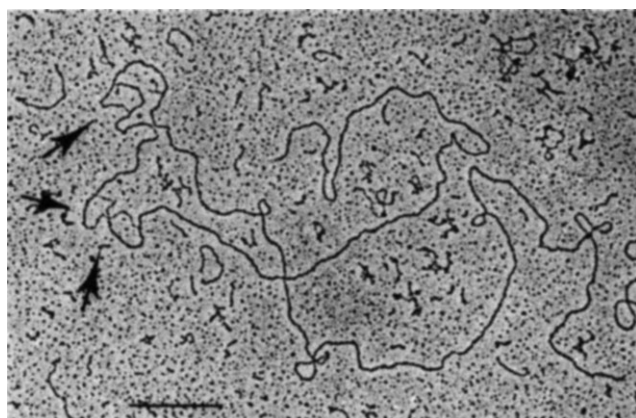


Fig. 5 Electron microscopic analysis of R-loop structures formed between intact recombinant DNA and early viral RNA. Recombinant DNA ($8 \mu\text{g ml}^{-1}$) and cytoplasmic RNA ($300 \mu\text{g ml}^{-1}$) from cells infected in the presence of cytosine arabinoside were incubated at 42°C for 20 h in a solution containing 70% formamide, 0.1 M Tricine buffer pH 8.0, 0.25 M NaCl and 0.01 M EDTA. Samples were mounted for electron microscopy by spreading the above mixture with no additions other than cytochrome *c* over a distilled water hypophase. Grids were rotary shadowed with platinum-palladium and examined in a Siemens Elmiskop 101 microscope at 40 kV accelerating voltage. Electron micrographs were taken on Kodak electron image plates at magnifications of 6,000. Magnification was calibrated with a grating replica (E. F. Fullam, cat. no. 1000), and contour lengths were measured with a Numonics digitiser interfaced to a Wang 2200 computer. R-loops are indicated by arrows, scale bar, $0.5 \mu\text{m}$. In this molecule, the possibilities that there are two R-loops, one of which is twisted, or three R-loops, two of which are adjacent, could not be distinguished. A schematic representation of the map positions of hybridised RNA is also presented. VAC indicates the position of the vaccinia virus DNA insert with the orientation of the original cross-linked end.

bands were resolved by agarose gel electrophoresis (track 1, Fig. 2). Starting at the top of the gel, the first three correspond to the reannealed λ arms and the individual large and small λ arms, respectively. Significantly, the fourth band co-migrated with authentic *EcoRI* end fragment obtained by *EcoRI* digestion of the larger (track 2, Fig. 2) or smaller (track 3, Fig. 2) *HindIII* end fragments of total vaccinia virus DNA.

To prove that the cloned DNA is identical to authentic *EcoRI* end fragment from vaccinia virus DNA, the insert was separated from the λ arms and cleaved with *HpaII*. The *HpaII* fragments obtained from the cloned insert (track 3, Fig. 3) co-migrated with common *HpaII* fragments obtained by sub-digestion of the two *HindIII* end fragments from vaccinia virus DNA (tracks 1 and 2, Fig. 3) as well as with *HpaII* fragments obtained by digestion of authentic *EcoRI* end fragment also isolated from vaccinia virus DNA (track 4, Fig. 3). In addition, since the largest *HpaII* fragment obtained from the insert is derived from the originally cross-linked end of the genome and since the mobility of this fragment is similar to that of the *HpaII* fragment containing the cross-link, relatively little DNA was lost by single-strand nuclease treatment prior to cloning. The orientation of the inserted vaccinia DNA was determined by mapping the single *XhoI* restriction site of the *EcoRI* end fragment of vaccinia virus DNA within the recombinant molecule. The recombinant used in these studies has the originally cross-linked end of the vaccinia virus genome attached to the large arm of the λ vector. However, a recombinant with the opposite orientation was also identified.

Expression of inverted terminal repetition

Preliminary results⁷ indicated that some sequences contained within the inverted terminal repetition are transcribed in vaccinia virus infected cells and further evidence for this was obtained by hybridisation of ³²P-labelled RNA to *HpaII* and *HincII* digests of *HindIII* fragments B and C from vaccinia virus DNA (E. B., unpublished). The cloned *EcoRI* fragment, however, provided a tool for isolating sufficient RNA to extend these studies. In particular, we wished to know whether RNA isolated by hybridisation to the cloned terminal repetition could serve as a messenger. Previously the message-dependent reticulocyte lysate, supplemented with calf liver tRNA, was shown to faithfully translate early and late vaccinia virus mRNA^{11,12}. In those studies we found that RNA made in the presence of an inhibitor of protein synthesis (referred to as immediate early RNA) and RNA made in the presence of an inhibitor of DNA synthesis (referred to as early RNA) programmed synthesis of similar sets of polypeptides although some differences were also noted. When translating RNA extracted from cells late after infection, a new set of polypeptides was made in addition to continued synthesis of some early polypeptides.

Figure 4 shows the virtually blank endogenous incorporation of the reticulocyte lysate (track 4) whereas tracks 1, 2 and 3 show polypeptides synthesised when the cell-free system was programmed with total immediate early, early and late mRNAs. Tracks 5, 6 and 7 show polypeptides synthesised when the cell-free system was programmed with mRNAs selected by hybridisation to the recombinant DNA immobilised on nitrocellulose membranes. In contrast to the large number of total

immediate early polypeptides, only three prominent polypeptides of MWs 42,000, 19,000 and 7,500 as well as several minor polypeptides were detected using selected immediate early mRNA (track 5). Similar polypeptides, but in lesser amounts, were also made with selected early mRNA (track 6). Longer periods of fluorography revealed synthesis of small amounts of the same three prominent early polypeptides using selected late RNA (track 7); however, no unique late polypeptides were detected.

Electron microscopy was used to visualise the early RNA species. Cytoplasmic RNA, from cells infected with vaccinia virus in the presence of cytosine arabinoside, was annealed to the recombinant DNA in conditions of high formamide concentration which favours RNA·DNA over DNA·DNA hybridisation¹³. Nearly all molecules contained at least one 'R-loop' formed by partial displacement of a DNA strand; most molecules contained two or three R-loops. From contour length measurements of the recombinant DNA, it was apparent that all R-loops were located within the vaccinia virus DNA insert. A photograph of one DNA molecule containing R-loops and schematic representations of the R-loop locations on 32 separate DNA molecules are shown in Fig. 5. To confirm the orientation of the R-loops within the vaccinia virus DNA insert, *HindIII* was used to cleave recombinant molecules within the short λ arm. A similar distribution of R-loops was observed in an additional 40 molecules. The R-loop (mean $0.28 \pm 0.07 \mu\text{m}$) nearest to the large λ arm (that is, the end of the vaccinia genome) was clearly observed to contain a short single-strand extension in 28 of 160 molecules. In each case the single-strand projection, presumably a poly (A) tail, was located on the side of the R-loop nearer to the long λ arm. As represented in Fig. 5, the R-loops nearer to the short λ arm were variable in size ($0.19\text{--}0.79 \mu\text{m}$; mean $0.45 \pm 0.17 \mu\text{m}$) and in apparent poly (A) tail position. However, in molecules where one large R-loop was visible, the appearance suggested that two adjacent mRNAs had been transcribed from opposite strands. Direct evidence for strand switching has recently been obtained (R. W. and J. C., unpublished) by hybridisation of RNA to separated recombinant DNA strands. Of some interest was the absence of any R-loops within the $2.08 (\pm 0.06) \times 10^6$ daltons corresponding to the very end of the viral genome. A similar conclusion was also reached by failure of ³²P-labelled early RNA to hybridise to *HpaII* and *HincII* terminal restriction fragments from virion DNA (E. B., unpublished). Accordingly, this region of the genome appears to be transcriptionally inactive. Another point of interest was our inability to visualise R-loop structures containing intervening DNA, suggestive of split genes, such as have been found for other DNA viruses.

Conclusion

We have shown that the inverted terminal repetition in vaccinia virus DNA is divided into transcriptionally active and inactive regions and thus appears to have more than one function. Besides a putative role in DNA replication, a portion of the terminal repetition codes for several early mRNA species. Although the genome organisation of herpesvirus is quite different from that of poxviruses, evidence for transcription of repetitive sequences in that system has also been found²⁴.

Received 26 November 1979; accepted 18 February 1980.

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LETTERS

Improved position and new optical candidate for A0538-66

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Among X-ray transients, A0538-66 is unique: between June and December 1977 it was observed to undergo seven outbursts¹⁻³, characterized by different durations (from ~12 h to ~15 days) and peak intensities (from ~0.03 to 0.1 Crab). Most remarkably, the outbursts were all separated by an interval of either 16.7 days or a small multiple thereof. The source may be a member of the Large Magellanic Cloud (LMC) and thus one of the most luminous stellar X-ray sources known [$(L_x(2-17 \text{ keV}) \approx 8 \times 10^{38} \text{ erg s}^{-1})$]. No steady counterpart to the flaring source has yet been detected. We report here a reanalysis of the HEAO 1 modulation collimator (MC) data which has led to the detection of the five outbursts which occurred between August and December 1977. A uniquely precise (25 arc s) position has been obtained which excludes the previously suggested optical candidate. We have searched archive plates of the LMC and find that one of the stars in the refined error region shows variability in the B band by ~1 mag.

The first two outbursts of A0538-66 were observed with the Ariel 5 satellite¹, leading to a position accurate to ~0.5°, and, for one of the outbursts, a spectrum best characterized by a thermal bremsstrahlung model with $kT = 6.5 \text{ keV}$ and no detectable low-energy absorption ($N_H < 2 \times 10^{22} \text{ cm}^{-2}$). Both of these outbursts lasted ≤ 1 day. After the launch of HEAO 1, the LMC was under nearly constant scanning surveillance due to its location at the south ecliptic pole. Two more brief (~1 day) outbursts were detected with the HEAO 1 MC in October and November 1977, resulting in three possible source locations within or near the Ariel 5 error region². Based on these four events, it was suggested that the outbursts showed a regularity in their onset that could be described by:

$$T_0 = (2443323.96 \pm 0.03) + (16.662 \pm 0.006)n$$

where T_0 is the Julian date of the onset time of the n th outburst². The Ariel 5 events correspond to $n = 0$ and 1, and the HEAO 1 MC observations to $n = 6$ and 8. A search for other brief outbursts in the HEAO 1 MC data was unsuccessful, and an upper limit of $0.9 \mu\text{Jy}$ was placed on the (1.5-13.5 keV) flux density of a steady component. A subsequent analysis of the HEAO 1 Large Area Sky Survey (LASS) data revealed that outbursts corresponding to $n = 3, 4$ and 11 did occur at the expected times³. The events at $n = 3$ and 4 lasted significantly longer than the other five; the flare at $n = 11$ occurred while LMC X-3 was unusually bright⁴.

The MC data for A0538-66, taken at maximum brightness according to the LASS light curve³, were superimposed about the centre of the previous most likely error region. (For a description of the instrument and data analysis technique, see ref. 5). For this optimum set of data, significant detections of all five outbursts were obtained (Table 1), yielding five pairs of lines of position spanning 150° in position angle. These lines have only one common intersection within ~2° of the Ariel 5 error region, thus confirming that all five outbursts, in spite of their different durations and peak intensities, originate from the same

source. The five pairs of lines were combined^{6,7} to yield the most likely source position:

$$\alpha(1950.0) 5 \text{ h } 35 \text{ min } 42.7 \text{ s}, \quad \delta -66^\circ 53' 44''$$

with a 90% confidence error circle radius of 25 arc s. A finding chart of the region is presented in Fig. 1a. The northern half of the previous most likely error region (region A of ref. 2), including the suggested optical candidate² (star 19 of Fig. 1a), is excluded by the refined position reported here.

We have made a crude search for X-ray spectral variability by computing the hardness ratio R for each detected outburst. This is defined as the ratio of the counting rates in the 5.4-13.3 keV to 0.9-5.4 keV energy bands. This ratio is not well determined for the flare at $n = 11$ due to the low statistical significance of the detection and to confusion with LMC X-3 in the lowest energy channel (0.9-2.6 keV). Three of the remaining four outbursts ($n = 3, 4$, and 6) are consistent with a constant hardness ratio, ($R = 0.51 \pm 0.05$; $\chi^2 = 1.8$ for 2 d.f.) and with the Ariel 5 spectral parameters. The outburst at $n = 8$ was significantly harder than the others: $R = 1.42 \pm 0.25$. Assuming negligible low-energy absorption, this corresponds to a change in the logarithmic slope of a power-law (energy) spectrum from -0.5 to +0.7. The data are, however, not sufficient to distinguish between a flattening of the source spectrum and an increase in the low-energy absorption. We note that the observed spectral change may complicate intensity comparisons between different instruments.

A search for optical variability or proper motion of the stars in the refined error region has been carried out with archival plate material at the Harvard College Observatory. The plates, taken with the 24" Bruce doublet at Bloemfontein, South Africa, were examined with a blink comparator, and the two showing the strongest evidence for variability were subsequently measured with an iris photometer. Nine nearby stars from the photometric sequence of Dachs⁸ were used for calibration. Star Q (Fig. 1a) is observed to be variable between extremes of $B \sim 14.5$ (12 November 1945; Plate no. A25156) and $B \sim 15.5$ (19 November 1941; Plate no. A22993), although the plates are not of sufficient quality for a detailed light curve to be compiled. Figure 1b shows the range of variability observed. Astrometry for stars C, D, Q and R has been carried out on plates taken 13 November 1896 (stars Q and R) and 19 November 1941 (stars C and D) and compared with the positions of the same stars on the ESO Quick Blue Survey plate (21 November 1973). Upper limits (3σ) of $0.28 \text{ arc s yr}^{-1}$ (stars C and D) and $0.15 \text{ arc s yr}^{-1}$ (stars Q and R) are placed on their proper motion, indicating that they are not very local objects⁹.

Spectra of stars Q and R were obtained 31 July 1979 (~5 days before the extrapolated flare onset time) with the RGO spectrograph and IPCS at the 3.8m Anglo-Australian telescope. Star R shows H and He absorption lines typical of a normal O star: we estimate $V = 13.8$, $B - V = -0.2$. Star Q shows the absorption spectrum of a B-type star: we estimate $V = 15.7$,

Table 1 Dates of observations used for position measurements

Outburst no. (n)	Dates (JD - 2443000)	Detection Significance		Average flux density* (μJy)
		MC1 (σ)	MC2 (σ)	
3	375.107-380.032	11.6	17.4	25
4	390.997-392.339	5.1	5.9	20
6	423.935-424.274	8.3	7.5	45
8	457.149-457.440	9.9	6.8	25
11	507.119-507.264	5.4	4.9	17

* For an assumed Crab-like spectrum averaged over 1.5-13.5 keV; $1 \mu\text{Jy} = 0.242 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$.

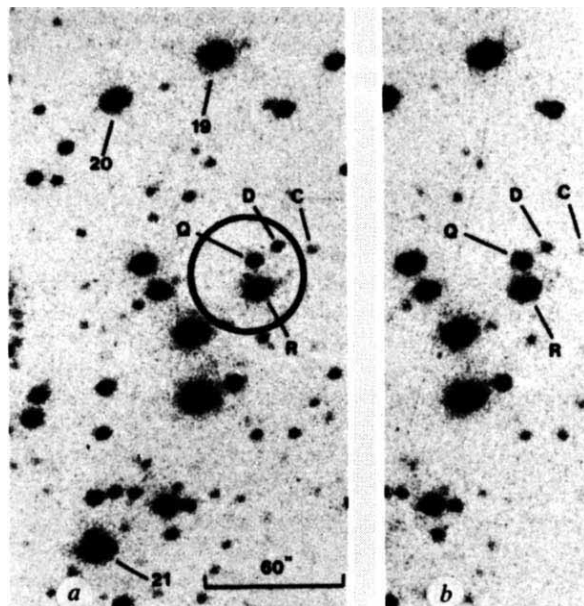


Fig. 1 Finding chart for A0538-66 prepared from Harvard College Observatory Plate no. A22993, taken 19 November 1941 with the 24-inch Bruce doublet in Bloemfontein, South Africa. (b), taken from Plate no. A25156 made with the same instrument on 12 November 1945, illustrates the variability of star Q by ~ 1 mag in the B band. The celestial location (± 2 arc s) of star Q is:

$$\alpha(1950.0) \ 5 \text{ h } 35 \text{ min } 42.4 \text{ s}, \quad \delta -66^\circ 53' 39''$$

Star numbers are from the photometric sequence of Dachs⁸.

$B - V = 0.0$, and conclude that it is a probable LMC member ($M_V = -3.8$ for an assumed distance modulus¹⁰ of 18.6 and foreground extinction $A_V = 0.2$ mag). We note the similarity of this star to the optical candidate for LMC X-3 ($V = 16.9$, $B - V = -0.06$, weak H β emission, variable by 0.1 mag; see ref. 4 and refs therein).

Transient X-ray phenomena on time scales of seconds (for example X-ray bursts; see review in ref. 11) and months (for example classical X-ray transients; see review in ref. 12) have led to optical identifications and extensive studies of the stellar counterparts. In contrast, sources with characteristic outburst times of hours to days have been observed¹³⁻¹⁷ but none have been positioned with sufficient accuracy for a convincing optical identification to be made (with the possible exception of H0449-55; ref. 17). Among sources exhibiting fast-transient behaviour, A0538-66 is of particular interest as its outbursts may have either hard or soft spectra, and may last hours or days. It thus spans the range of behaviour observed in other fast-transient sources, which, while possibly recurrent, are not located in a region of the sky subject to as continuous a surveillance as the LMC during the HEAO 1 mission. Several possible explanations for the behaviour of this source were considered in ref. 2, but present data do not significantly constrain possible source models. Optical and radio monitoring of star Q, especially near the expected times of X-ray outbursts, will be important in establishing variability commensurate with the X-ray periodicity and in elucidating the nature of this unusual system.

We thank E. Ralph for the plate search for optical variability and for preparing the finding chart; M. Conroy, M. Garcia, and W. Roberts for assistance in the X-ray data analysis; M. Smith for assistance in taking the optical spectra; and G. Skinner, S. Shulman, and A. Szymkowiak for results before publication. R.E.G. thanks the Smithsonian Institution, and M.J.W. the SRC for travel support. We also acknowledge the assistance of the staff of the Anglo-Australian Observatory. The work was supported in part by NASA under contracts NAS8-30543 and NAS8-27972.

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Freeze-formed silica fibres

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Phase separation during directional freezing of liquid systems leads to a variety of microstructures of the components due to the interplay of heat transfer, diffusion kinetics, and interfacial surface energy¹⁻⁶. One of the more dramatic effects is the *in situ* formation of parallel fibres in directionally solidified eutectic superalloy. We report here similar morphological consequences of freezing aqueous polysilicic acid. This system is unique not only because it has an amorphous component, polysilicic acid, but this substance undergoes accelerated, concentration-dependent polymerization immediately after phase separation. As a result, it becomes insoluble, and after thawing, preserves the structure that had been conferred on it by the surrounding ice. We have explored the relationships of polymerization, composition, and freezing conditions with the morphology of the products, in particular with regard to fibre formation.

A solution of silicic acid in water (100 ml, 1 M in SiO₂) was prepared by ion exchange⁷, adjusted to pH 5 with 0.3 ml 1 M NH₄OH, and placed in a 24 $\times$ 2.7 cm polypropylene cylinder. The silicic acid, which gelled 15 min after adjusting the pH, was allowed to age for another 30 min and was then directionally frozen by lowering the plastic cylinder into a -70 $^\circ$ C cold bath at 4 cm h⁻¹. After thawing, all the silica was in the form of insoluble parallel fibres with polygonal cross-section, ~ 0.005 cm in diameter and 15 cm long (Figs 1, 2). Fibres have been obtained from gels of this type with freezing rates from 0.3 to 150 cm h⁻¹ and bath temperatures from -10 $^\circ$ C to -196 $^\circ$ C (ref. 8). Average fibre diameter decreases with lower silicic acid concentration, colder freezing baths, or with faster freezing rates in agreement with theoretical treatments of eutectic alloys¹⁻⁶.

Although each fibre has a unique cross-sectional geometry, the cross-section is invariant along the length of the entire fibre when freeze-formed in uniform conditions. When a cylinder of gelled silicic acid is frozen statically by cooling one end, both the thermal gradient and the freezing rate decrease as the ice-gel interface advances⁹. In these conditions, the resulting fibres are tapered (for example, 0.004 cm diameter at one end, and 0.026 cm at the other end of an 11-cm long fibre).

After drying in air at 150 $^\circ$ C, the composition of the fibres corresponds to Si₃O₅(OH)₂. The fibres are amorphous (X ray) and porous; surface area is about 900 m² g⁻¹, and the density measured by flotation in carbon tetrachloride-bromoform is 1.99 g cm⁻³. The tensile strength is 86 $\pm$ 35 MPa. After heating at 925 $^\circ$ C for 8 h, the composition corresponds to SiO₂; surface area is decreased, the density is 2.20 g cm⁻³, and the tensile strength is 510 $\pm$ 216 MPa. After 8 h at 1,000 $^\circ$ C, the average tensile strength drops to 253 MPa, and incipient crystallization can be detected; except for shrinkage, the fibre geometry remains unchanged.

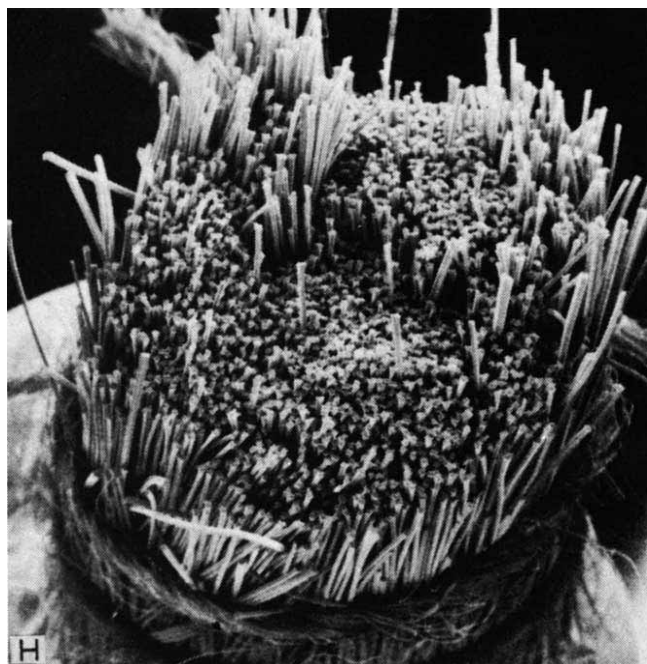


Fig. 1 Scanning electron micrograph of a bundle of silica fibres bound with twine and cut with scissors. Scale bar, 100 μm . Magnification $\times 20$.

The slower polymerization rate of 1 M silicic acid at pH 3 (gel time is 24 h at 25 $^{\circ}\text{C}$) permits a wider range of product morphologies to be prepared. Fresh silicic acid, frozen and thawed promptly, reforms soluble silicic acid¹⁰. After ageing 1 M silicic acid 1 day at 25 $^{\circ}\text{C}$, freezing and thawing gives insoluble imbricated flakes, roughly 0.1 cm across with parallel ridges 0.001–0.002 cm apart on one side (Fig. 3). The same morphology is observed when fresh silicic acid is frozen, aged 1 day at -10°C , and then thawed. After 3 days' ageing at 25 $^{\circ}\text{C}$ before freezing, flakes are obtained that are accompanied by honeycomb structures and 6 days' ageing before freezing gives polygonally cross-sectioned fibres having about 550 $\text{m}^2 \text{g}^{-1}$ surface area after drying. After ageing for about 20 days, fibres are

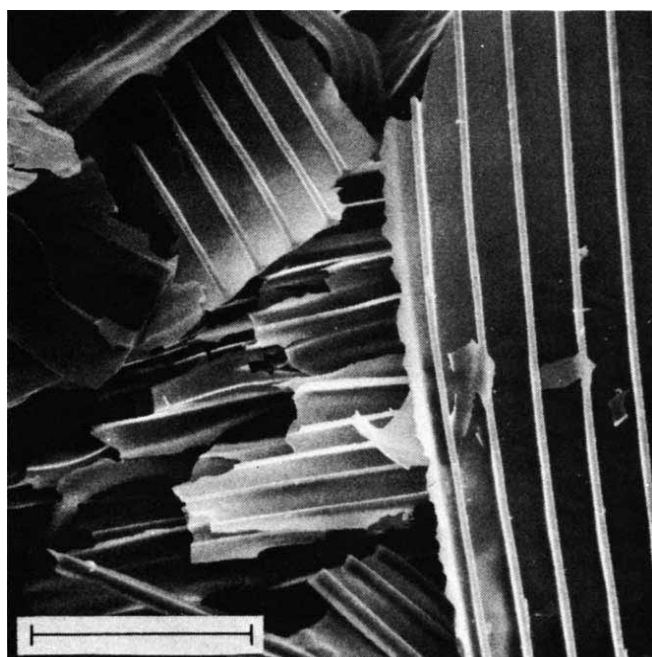


Fig. 3 Ribbed flakes. Scale bar, 100 μm . Magnification $\times 300$.

Freezing of silicic acid and other colloids has been widely discussed, but all morphological descriptions of the products have been of small particulates, specifically powders¹¹, flakes^{12,13}, ribbed flakes¹⁴, interconnected cells¹⁵, and granules^{16,17}. Our finding that high gel strength, low electrolyte concentration, and unidirectional freezing seem to be essential for obtaining polygonally cross-sectioned fibres may have some generality. Zirconia gels prepared by dialysis have responded similarly.

We thank M. L. Van Kavelaar for the scanning electron microscope pictures. Contribution 2730 from the Central Research and Development Department, E.I. du Pont de Nemours & Company.

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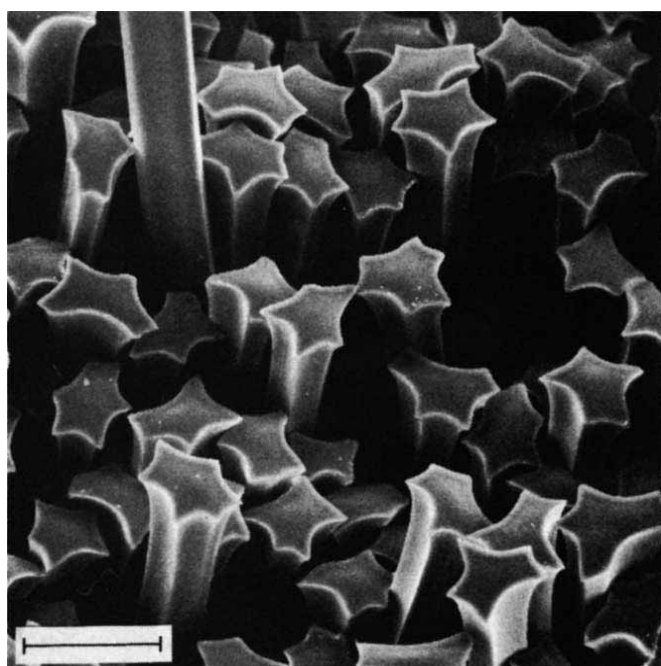


Fig. 2 Silica fibres. Scale bar, 100 μm . Magnification $\times 200$.

A thin-film polycrystalline photoelectrochemical cell with 8% solar conversion efficiency

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Photoelectrochemical solar cells (PECs) may become an economic method for conversion of light to electricity, due largely to the simplicity of formation, and quality, of the polycrystalline semiconductor-electrolyte junction. Within the past few years, conversion efficiencies of thin-layer polycrystalline-based cells have increased from ~1% to a recently reported 7.3% for the n-GaAs/Se₂⁻ system¹. PECs based on the CdSe-polysulphide system have been studied extensively recently. CdTe, with a band-gap, E_g , ~1.45 eV is better matched to the solar spectrum than CdSe (E_g ~1.75 eV), but is unstable in polysulphide solutions as a photoanode at the current densities to be expected under normal solar irradiation (>10 mA cm⁻²). A polysulphide-based PEC is described here which uses polycrystalline layers of CdSe_{0.65}Te_{0.35}, electrodes of which are prepared by painting a slurry of the semiconductor onto a Ti substrate, and sintering. Subsequent surface treatments of these layers lead to a solar-to-electrical conversion efficiency of up to 8%, with stability comparable to the thin-layer polycrystalline CdSe/polysulphide system².

CdSe and CdTe form solid solutions over the entire composition range. The electrical, optical and thermoelectrical properties of these alloys have been studied, and a minimum in the band gap of ~1.35–1.45 eV (depending on crystal structure) has been found³. To use these alloys as photoelectrodes in liquid junction photovoltaic cells layers of the alloys were prepared by a process similar to screen printing, whereby a CdSe_{0.65}Te_{0.35} powder (prepared by sintering CdSe and CdTe powders

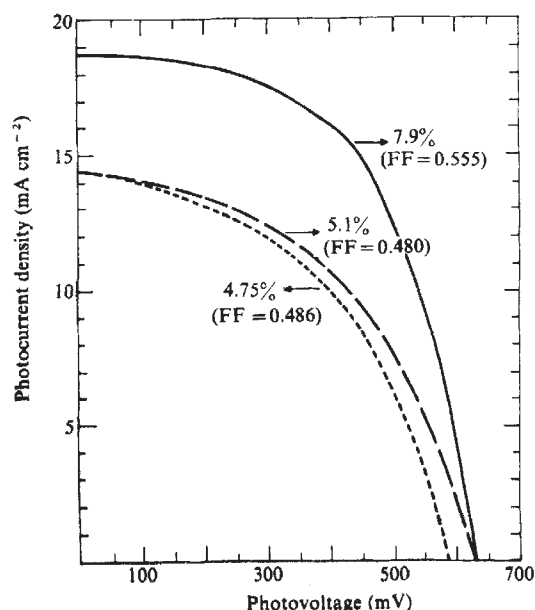


Fig. 1 Photocurrent-photovoltage characteristics for polycrystalline CdSe_{0.65}Te_{0.35} electrodes in polysulphide solution (1 M each KOH, Na₂S, S) under 0.85 X AMI simulated sunlight. . . ., after HCl:HNO₃ etch, electrode area 0.22 cm²; ---, after CrO₃ etch or K₂CrO₄ treatment; —, after photoetch + K₂CrO₄ treatment, electrode area 0.55 cm².

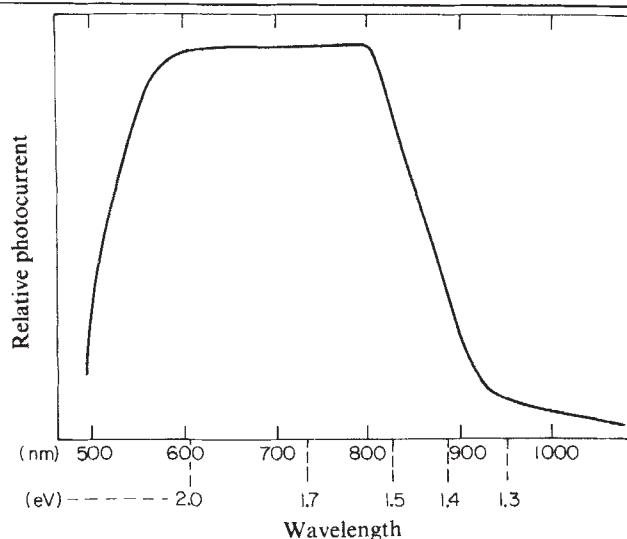


Fig. 2 Spectral response of a polycrystalline CdSe_{0.65}Te_{0.35} photoanode in polysulphide solution (1 M each KOH, Na₂S, S). The photocurrent is corrected for photon density (that is the spectrum compares the relative quantum efficiency with the wavelength). The shape of this response, together with X-ray diffraction data, which shows that these electrodes are of a single composition, and with lattice parameters to be expected from this composition (compared with those of ref. 6), proves that the semiconductor is indeed a homogeneous alloy.

together with a CdCl₂ flux) was made into a smooth aqueous paint together with CdCl₂, painted onto a Ti substrate and then annealed in an inert atmosphere. The resulting electrodes were etched, and tested in an aqueous electrolyte containing 1 M each of KOH, Na₂S and S, with a counter electrode of sulphide brass gauze⁴. The light source was either solar illumination, or a tungsten source calibrated for the CdSe_{0.65}Te_{0.35} cells against solar illumination.

The performance of the photoelectrodes depends strongly on the etching treatment used. Figure 1 shows examples of the output characteristics for three different treatments. The dotted line represents output characteristics obtained after a HCl:HNO₃ etch (the usual etch for CdSe photoelectrodes). Solar efficiencies up to 5.0% have been obtained after this treatment. Etching in dilute aqueous CrO₃ leads to an improved open circuit voltage and solar efficiencies up to 5.5%. The same increase of the OCV can be obtained after the HCl:HNO₃ etch by treating the electrode surface with a solution containing CrO₄²⁻ ions before immersion in the polysulphide electrolyte.

A major increase in both short circuit current and fill factor (FF) was obtained by a subsequent photoetch, whereby the photoelectrode, shorted to a carbon counter electrode, was illuminated in a dilute aqueous mineral acid solution, for example, 0.1 M H₂SO₄. After treatment with an aqueous K₂CrO₄ solution, solar efficiencies up to 8.0% were obtained (the solid line in Fig. 1 shows the performance of a CdSe_{0.65}Te_{0.35} photoelectrode after such a treatment).

Figure 2 shows the spectral response for a CdSe_{0.65}Te_{0.35} photoelectrode in 1 M each of KOH, Na₂S and S. The short wavelength cutoff can be attributed to absorption by the polysulphide electrolyte. As the photocurrent loss due to this absorption is the same for CdSe and the CdSe_xTe_{1-x} system, the fractional loss in the case of CdSe (E_g ~1.75 eV) is considerably greater than for the smaller band-gap mixed compounds. This explains the much greater photocurrents obtained for CdSe_{0.65}Te_{0.35} than for CdSe (~60% increase was typically found), compared with what would be expected from the difference in band gaps, calculated on the basis of photon intensity of the relevant sections of the solar spectrum (<40% increase). The plateau region is surprisingly flat, and the long

wavelength cutoff is fairly sharp (considering the polycrystalline nature of the electrode), and shows an effective band gap of ~ 1.45 eV, similar to that of CdTe.

The output stability of the photoelectrodes in polysulphide electrolyte has been measured in various conditions. In an electrolyte 3 M each in KOH and Na_2S and 4 M in S, and at 35°C , the photocurrent remained stable for 21 h at 20 mA cm^{-2} . After 21 h the illumination intensity was increased to give a photocurrent density of 28 mA cm^{-2} . The photocurrent decreased 3.6% (to 27 mA cm^{-2}) after a further 20 h. Another electrode, illuminated to give an initial photocurrent density of 42 mA cm^{-2} , dropped 16.8% in current output after 49 h. Note that the normal operating currents of such photoelectrodes would be less than 20 mA cm^{-2} . It has been shown that for CdSe operating in a polysulphide electrolyte, complete stability may be obtained for a certain amount of charge passed at low photocurrent densities, while at higher current densities, a large degree of instability may be manifested for the same amount of charge passed⁵. This is a consequence of the exchange between solution sulphide and selenium from the CdSe, which depends on the relative rates of the competing S^{2-} oxidation and CdSe photodecomposition reactions^{2,5}. The same mechanism is to be expected at the mixed $\text{CdSe}_x\text{Te}_{1-x}$ electrodes. Therefore the actual stability of these electrodes in normal (solar) operating conditions will be considerably better than suggested by the above 'worst case' conditions.

The effect of the photoetching treatment has been found to be general for many semiconductors besides $\text{CdSe}_{0.65}\text{Te}_{0.35}$. Scanning electron micrographs of the semiconductor surface show extensive pitting of the surface by the photoetch, which therefore seems to act as a selective etch. The increased surface area resulting is clearly visible to the eye as a change from a dull grey surface to a velvet matt black one. Part of the increase in photocurrent after photoetching is probably due to a lowered reflectivity of the surface, but this cannot explain it completely, as the original surface was itself not very reflecting ($\sim 10\%$ reflectivity). Also, the increase in the fill factor, typically by $\sim 10\%$, remains to be explained. The photoetching effect and the effect of the composition of the $\text{CdSe}_x\text{Te}_{1-x}$ electrodes on their output stability will be discussed in detail elsewhere.

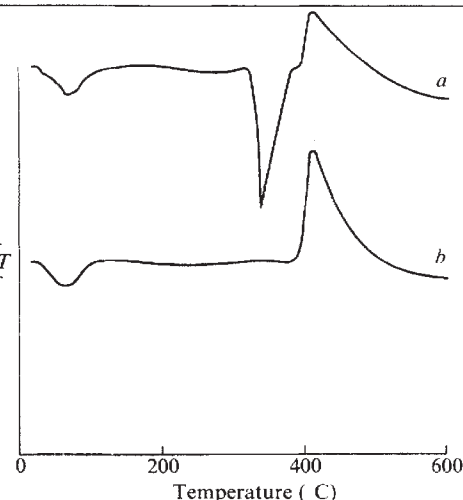


Fig. 1 Differential thermal analysis in one atmosphere oxygen of: *a*, precursor to ZSM-5 prepared from the NH_4^+ -TPA system; *b*, precursor to ZSM-5 prepared from the Na^+ -TPA system. The small endotherm at $\sim 100^\circ\text{C}$ is due to the loss of absorbed water. The endotherm at $\sim 340^\circ\text{C}$ and exotherm at $\sim 410^\circ\text{C}$ are due to the decomposition of the NH_4^+ ion and the oxidation of TPA decomposition products respectively.

occluded into the structure to give precursors to the zeolites. Because of the channel size the organic ions can be removed only by thermal degradation, giving the zeolites. When the precursors are synthesised from the Na^+ -TAA system, Na^+ ions remain in the zeolite for charge compensation. These must be exchanged by conventional ion exchange procedures if the H-form of the zeolite is required. This form has considerable potential for catalysing the conversion of oxygen-containing organic compounds, particularly methyl alcohol, to hydrocarbons^{8,9}.

For our present experiments we used NH_4^+ -TAA systems which produce precursors containing NH_4^+ ions for charge compensation. When the precursors are heated to remove the occluded organic ions the NH_4^+ ions also decompose, giving a direct route to the H-form of the zeolite. This is shown by differential thermal analysis (Fig. 1). As the temperature is increased, the initial reaction is the decomposition of the NH_4^+ ion to give NH_3 and H^+ . This is followed by decomposition of the TAA to give the H-form of the zeolite directly. A further advantage of NH_4^+ -TAA systems is that the reaction stops with the formation of the zeolite, whereas in Na^+ -TAA systems the zeolite is only an intermediate and the final reaction product is α -quartz. Crystals produced in the NH_4^+ -TAA systems differ slightly in morphology from those produced using Na^+ -TAA systems^{1,2}. The ZSM-5 crystals are often twinned. The ZSM-11 crystals are the same ovate shape as are those produced in the Na^+ -TAA system, but are about five times longer, being up to $10\text{ }\mu\text{m}$ long.

A typical preparation for H-ZSM-5 is: SiO_2 , 0.0655 M; Al_2O_3 , 0.000205 M; TPA hydroxide, 0.008 M; NH_4OH , 0.15 M; H_2O , 1.0 M; heated at 160°C under autogeneous pressure for 3 days. The SiO_2 has to be in an active form, such as silicic acid or colloidal SiO_2 , and Al_2O_3 can be supplied as $\text{Al}(\text{OH})_3$. When low Na-content reagents are used in the NH_4^+ -TAA system, H-ZSM-5 and H-ZSM-11 can be prepared containing 1 p.p.m. Na. We have found approximately the same reaction rates and product yields in these Na^+ -free NH_4^+ -TAA systems and in the Na^+ -TAA systems, and do not believe that Na^+ plays an essential part in the NH_4^+ -TAA systems.

Other zeolites which have been synthesised in single-base systems free of alkali metal ions are analcite, faujasite, type A and harmotome in systems containing NH_4OH only or tetramethyl ammonium (TMA) only¹⁰, and sodalite¹¹ and gismondine¹² in systems containing TMA only. However, the Na^+

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NH_4^+ -tetraalkyl ammonium systems in the synthesis of zeolites

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ZSM-5 (ref. 1) and ZSM-11 (ref. 2) are silica-rich aluminosilicate zeolites with similar two-dimensional intersecting channel structures. They are conventionally synthesised from Na^+ -tetraalkylammonium (Na^+ -TAA) systems at high pH and temperatures around 160°C (refs 3-6). We report here what we believe to be the first use of systems free of alkali metal ions in the synthesis of the zeolites ZSM-5 and ZSM-11.

In the conventional synthesis other alkali metal ions can be used but we have found that the rate of reaction is highest with Na^+ . Tetraethyl ammonium (TEA) or tetrapropyl ammonium (TPA) give ZSM-5, while tetrabutyl ammonium (TBA) forms ZSM-11. The TAA ions probably act as templates⁷ and are

contents of these systems were not reported, and may have been of significance as it has been found¹³ that 'trace' amounts of Na are necessary to initiate crystallisation of some zeolites.

Silicalite-1¹⁴ and silicalite-2¹⁵, the aluminium-free analogues of ZSM-5 and ZSM-11 respectively, differ from the zeolites in that no charge compensating cations are incorporated into the structure. Their syntheses differ in some minor respects from those of the zeolites. We find that the silicalites can be prepared using TAAs only, although the reaction proceeds very slowly. However, we have had no success in preparing the zeolites in the presence of TAAs only. Silicalite-2 cannot be prepared in the presence of any alkali metal ion, although it is claimed that the corresponding zeolites can be synthesised using the Na⁺-TBA system⁵.

Note that ZSM-5 can also be produced using the Na⁺-primary amine system¹⁶. However, when the Na⁺ is replaced with NH₄⁺ the rate of reaction is very much less and it is possible that trace amounts of Na⁺ (~0.01%) are catalysing the reaction.

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Clinoenstatite in boninites from the Bonin Islands, Japan

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Highly magnesian andesitic lavas were considered a rare and unusual rock type, before boninites proved to be so extensive among the Tertiary volcanic rocks in the Bonin-Mariana region¹⁻⁵ and some ophiolitic basalts^{6,7}. Protoenstatite is also a rare mineral and its terrestrial occurrence has been confined to the high-Mg andesites from Cape Vogel, eastern Papua⁸, and the Mariana Trench³. Some boninites from the Bonin Islands were found to contain abundant multiply-twinned clinoenstatite (inverted protoenstatite) with or without olivine. The clinoenstatite, coexisting with olivine with a similar forsterite content to that in the mantle, shows that protoenstatite crystallized later than the olivine, whereas in the olivine-free, clinoenstatite-bearing rock which has more SiO₂ than the clinoenstatite-bearing or -free boninites with olivine, the protoenstatite may have been the liquidus mineral. We show here that the presence of inverted protoenstatite in the Bonin Islands confirms our earlier suggestion^{1,9,10} that boninites may have been formed by extensive partial melting of hydrous peridotite at relatively low pressures and rapid quenching from high temperatures at shallow depths.

The Bonin or Ogasawara Islands, which form the outer arc of the Idu-Mariana island arc in the Western Pacific, comprise three island groups: Chichi, Haha and Muko, which consist predominantly of volcanic rocks of Eocene to Oligocene age. The central Chichi-jima group is composed of a sequence of lower boninite pillow lavas and upper andesite or dacite breccias, on which late Oligocene limestone rests unconformably. The Haha-jima group, lying some 50 km south of Chichi-jima, has a basalt-andesite-dacite suite with tholeiitic affinities of Eocene age, but boninite has never been found². We have shown¹¹ that Muko-jima, ~70 km north of Chichi-jima, is made up largely of boninite pillow lavas and breccias containing occasional clinoenstatite. We found pillow lavas having more than 5 modal % clinoenstatite from Uguisu-hama, Muko-jima; Yoake-daira, Chichi-jima; and Kasa-yama, Chichi-jima. Komatsu also reported¹² some clinoenstatite-bearing boninites from Chichi-jima and Muko-jima.

The pillow lava from Uguisu-hama of Muko-jima contains abundant, large, dull white phenocrysts of clinoenstatite up to 10 cm long in the central part of the pillow, together with bright green bronzite phenocrysts up to 3 cm long. Olivine is very rarely present, having completely altered to pale brown or green products and carbonates. The rim is glass-rich and fine-grained; most phenocrysts are <1 mm in size. Its mineral proportions are: (modal %) clinoenstatite phenocrysts 1, orthopyroxene phenocrysts and microlites 16, clinopyroxene (augite and pigeonite) microlites 35, and glass 48. The whole rock analysis (Table 1, column 1) shows high SiO₂ content relative to clinoenstatite-free boninites (Table 1, columns 3 and 4). Electron probe analyses of the clinoenstatite phenocrysts ($\alpha_{\min} = 1.660$; $2V_x = 38-48^\circ$) reveal the highest Mg/Fe ratio and the lowest CaO, Al₂O₃ and Cr₂O₃ content in those from the boninites, whereas the orthopyroxene phenocrysts ($2V_x = 71-94^\circ$) have nearly the same composition as other boninite orthopyroxenes (Table 2 and Fig. 1). CaO, Al₂O₃ and Cr₂O₃ contents in the clinoenstatite increase with decreasing Mg/Fe ratio.

Table 1 Wet chemical analyses (wt %) of clinoenstatite-bearing and -free boninites from Bonin Islands (recalculated anhydrous)

	1	2	3	4
SiO ₂	59.04	56.65	56.70	55.99
TiO ₂	0.20	0.20	0.25	0.23
Al ₂ O ₃	11.47	11.54	13.22	10.47
Fe ₂ O ₃	1.65	2.36	0.66	2.65
FeO	7.21	6.57	6.83	6.66
MnO	0.30	0.22	0.17	0.17
MgO	11.18	14.33	12.58	15.64
CaO	6.99	6.99	6.79	6.77
Na ₂ O	1.38	0.85	2.00	1.16
K ₂ O	0.56	0.27	0.72	0.24
P ₂ O ₅	0.01	0.01	0.06	0.01
Cr (p.p.m.)	600	1150	840	1240
Ni (p.p.m.)			320	
Rb (p.p.m.)		10	8	0.5
F (p.p.m.)	120		110	140

1, Clinoenstatite-bearing rock, rim of pillow, Uguisu-hama, Muko-jima.

2, Clinoenstatite-bearing boninite, pillow breccia, Kasa-yama, Chichi-jima.

3, Clinoenstatite-free boninite, centre of pillow, Tsuru-hama, Chichi-jima.

4, Olivine-bronzite andesite, centre of dyke, Hatsune-ura, Chichi-jima.

The boninites from Yoake-daira and Kasa-yama of Chichi-jima have similar general petrographic characteristics. Both rocks contain prominent phenocrysts of both olivine and clinoenstatite together with orthopyroxene phenocrysts. The phenocrysts are set in a groundmass composed of ortho- and clino-pyroxene microlites and glass.

The clinoenstatite-bearing boninite from Yoake-daira is the most olivine- and clinoenstatite-enriched so far observed, consisting of 10% olivine, 11% clinoenstatite, 29% orthopyroxene, 25% clinopyroxene and 25% glass and others. The olivine phenocrysts of 0.5-3 mm across are consistently more magnesian (Fo_{90-91.5}) than those from the clinoenstatite-free boninites (Fo_{87-89.5}) (ref. 13); their $2V_x$ values are high, ranging

Table 2 Representative electron probe analyses for clinoenstatite, bronzite and olivine in clinoenstatite-bearing boninites from Bonin Islands

	1	2	3C	3M	4C	4M	5E	5B	6E	6B
SiO ₂	58.10	58.31	57.79	56.81	40.46	40.49	58.05	57.51	56.78	55.88
TiO ₂	0.00	0.00	0.01	0.01	0.00	0.00	0.02	0.01	0.01	0.00
Al ₂ O ₃	0.12	0.17	0.36	0.56	0.04	0.01	0.42	0.57	0.52	0.84
Cr ₂ O ₃	0.29	0.22	0.32	0.37	0.12	0.08	0.41	0.68	0.35	0.59
FeO	5.25	5.29	5.95	7.40	9.29	9.63	5.96	6.89	7.96	9.19
MnO	0.12	0.07	0.14	0.16	0.15	0.18	0.18	0.22	0.19	0.22
NiO	0.02	0.02	0.06	0.05	0.34	0.27	0.06	0.03	0.15	0.02
MgO	35.75	35.82	35.10	34.16	49.50	49.36	34.84	33.60	32.71	31.27
CaO	0.19	0.22	0.32	0.42	0.09	0.14	0.33	0.95	0.67	1.54
Na ₂ O	0.01	0.03	0.00	0.01	0.02	0.00	0.01	0.01	0.02	0.08
K ₂ O	—	0.01	—	—	—	—	—	—	—	—
Total	99.85	100.16	100.05	99.95	100.01	100.16	100.28	100.47	99.36	99.63
Mg/(Mg+Fe)	0.924	0.924	0.913	0.892	0.905	0.902	0.912	0.897	0.880	0.858
Mg	92.1	92.0	90.8	88.4	90.4	90.0	90.7	88.1	86.9	83.3
Fe	7.6	7.6	8.6	10.8	9.5	9.8	8.7	10.1	11.9	13.7
Ca	0.3	0.4	0.6	0.8	0.1	0.2	0.6	1.8	1.3	2.9

1, Large clinoenstatite phenocryst, centre of pillow, Muko-jima. 2, Clinostatite phenocryst, rim of pillow, Muko-jima. 3C, M, Core and margin of rim clinostatite around olivine (analysis 4), Yoake-daira. 4C, M, Core and margin of olivine adjacent to clinostatite (analysis 3), Yoake-daira. 5E, B, Clinostatite and bronzite of composite crystal, Yoake-daira. 6E, B, Clinostatite and bronzite of composite crystal, Kasa-yama. Mineral analyses were done on a JXA-5A electron microprobe at Nagoya University.

from 89 to 97°, compared with other boninite olivines ($2V_x = 86-93^\circ$) (ref. 10). Clinostatite occurs as: (1) discrete phenocrysts usually 0.5–3 mm long, rarely reaching 1 cm; (2) rims around the olivine phenocrysts; and (3) composite crystals with bronzite. Some of the phenocryst clinostatites have higher Mg/Fe ratio than the coexisting olivine phenocrysts, although the rim clinostatites are generally lower in Mg/Fe than the olivine. Note that the Mg/Fe ratio is higher in a core (Table 2, column 3C) of a large rim clinostatite (0.5 mm across) than in the adjacent olivine (Table 2, column 4), whereas it is lower at the margin (Table 2, column 3M) immediately adjacent to the olivine. In the composite crystals clinostatite is richer in Mg and Si, and poorer in Fe, Mn, Ca, Al, and Cr than bronzite, as already shown^{8,14}. The rock includes the most magnesian orthopyroxene ($2V_x = 101^\circ$; Fig. 1) analysed in the boninites.

The clinostatite-bearing boninite from Kasa-yama is less olivine- and clinostatite-enriched, having 4% olivine and 7% clinostatite. The size of those phenocrysts is small, mostly <0.5 mm. No fresh olivine was observed. The clinostatite

($\alpha_{\min} = 1.661$) is the least magnesian among the three clinostatite-bearing rocks. The whole rock chemical composition (Table 1, column 2) is not very different from that of the clinostatite-free typical boninite, although it may be slightly higher in SiO₂ at a given MgO content.

In the clinostatite-bearing boninites of Chichi-jima the distribution of Mg and Fe between the olivine and rim clinostatite suggests that the latter formed by reaction of olivine and magma, although some of the phenocryst clinostatite may have crystallized almost simultaneously with olivine. On the other hand, for the clinostatite-bearing rock of Muko-jima in which olivine is virtually absent, the large clinostatite phenocrysts richest in Mg seem to have been the liquidus phase instead of olivine. The crystallization of the clinostatite (protoenstatite) may have been promoted by the high SiO₂ content in the magma and/or the release of water from the water-saturated magma at a shallow depth. The clinostatite-bearing boninites would be generated at higher temperatures than the clinostatite-free boninites.

All the clinostatite-bearing rocks from the Bonin Islands, Cape Vogel and the Mariana Trench have high MgO contents, >11 wt %. Judged by the experimental studies^{15,16}, high temperatures at least 1,200 °C at relatively low pressures (<15 kbar) would be required to produce such highly magnesian and siliceous liquids even in water-saturated conditions. In many island arcs it seems unlikely that temperatures above or near the descending slab are high enough to generate the clinostatite-bearing rocks. A possible site may be a spreading ridge or an island arc with a steep geothermal gradient by previous magma generation where water liberated from the underlying subducted oceanic crust can be introduced.

We thank Professor K. Ishioka for helpful comments.

Received 5 December 1979; accepted 27 February 1980.

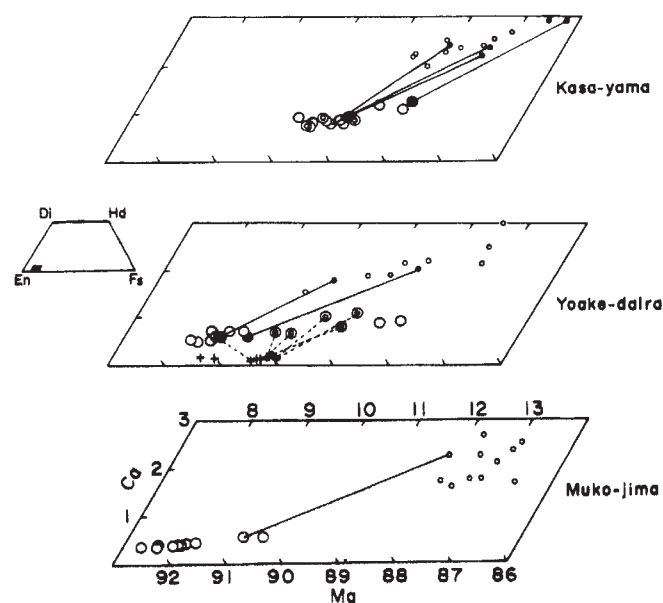


Fig. 1 Compositions of phenocryst clinostatites (○), rim clinostatites (◐), composite crystals (●), orthopyroxenes (◻), and olivines (+) in clinostatite-bearing boninites plotted on the pyroxene quadrilateral. Adjacent grains are connected by lines.

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Palaeozoic plants from Saudi Arabia

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A fossil flora recently discovered in the central part of Saudi Arabia is reported here. This represents the first assemblage of plants of Permo-Carboniferous age in the Arabian peninsula. The assemblage is of interest in showing affinity with contemporaneous northern Euramerian floras rather than those of Cathaysia or Gondwanaland to the east and south of Arabia.

More than 1,000 specimens were collected from an exposure alongside the Unayzah to Buraydah road, in the town of Unayzah (Fig. 1). The plants are preserved as impressions in a 5–20-cm limonitic shale band within a sequence of beds of alternating sand and shale, close to the base of the Khuff Formation. The Khuff has been tentatively assigned to the Upper Permian on faunal evidence; the Lower Khuff fauna was collected between 40 and 72 m above the base of the Khuff and that of the Upper Khuff, collected from 2 to 28 m below the top of the formation¹.

No rocks of Carboniferous or early Permian age have yet been recognized as occurring in outcrops in the Arabian peninsula, although strata dated as Carboniferous on palynological evidence have been recognized² in sub-surface occurrence. Powers *et al.*¹ reported *Lepidodendron* and *Dadoxylon* as having been recorded from outcrop of the Khuff Formation, but no documentation for these records or other details are given. Carboniferous beds with plant fossils are known from Syria³ and Iran⁴.

The most abundant plant in the assemblage from Unayzah (occurring on approximately two-thirds of the rock specimens seen) is a species of *Pecopteris* (Fig. 2f). Some of this material bears sporangia (Fig. 2c) and these indicate an affinity with the *Dizeugotheca-Acitheca* complex. Fronds of similar aspect (*Pecopteris hemiteloides*, *P. unitus*) are widely known from European and Cathaysian late Carboniferous and Permian floras, but also occur in some of the so-called mixed floras of Gondwanaland (for example, that at Wankie, Rhodesia⁵).

Of similar abundance to the *Pecopteris* are strap-shaped leaf fragments up to 5 cm broad, with fine parallel venation, resembling *Cordaites principalis* (Fig. 2a). This species occurs widely in the Northern Hemisphere late Carboniferous and early Permian. The third most abundant species is *Annularia stellata* (Fig. 2b). This species of calamite foliage is reported widely in North America and Europe, and also from the Cathaysian flora^{6,7}. The leaves are evenly spaced in their whorls, unlike the characteristically Cathaysian genus *Lobatannularia*, which has a distinct segregation of leaves of each whorl into two opposed groups. The Arabian leaves show features of structural detail which uniquely characterize this species; a broad strip of longitudinal striations overlies the midrib, and is flanked by broad zones of transverse striations⁸.

In addition, there are other fern-like leaves, such as *Validopteris*, with pinnae about 20 mm broad. Other pinnae of similar dimensions are determined, as cf. *Marattiopsis* (Fig. 2d,e). Attempts to extract spores from the Unayzah matrix and attached sporangia have failed.

The plants from Unayzah suggest an age not greater than Westphalian (late Carboniferous), particularly on the association of *Annularia*, *Cordaites* and the abundant *Pecopteris*, and the occurrence of *Annularia stellata* further suggests that the flora is not younger than early Permian. *Marattiopsis*, however, has recently been described from the late Permian of eastern

USSR⁹ and this genus is otherwise only recorded from Triassic and younger Mesozoic rocks in which it is a characteristic element of northern floras. The plant assemblage described here, taken as a whole, favours an age between late Carboniferous and early Permian; if this age is sustained by further evidence, this Arabian *Marattiopsis* will constitute the oldest record of the genus.

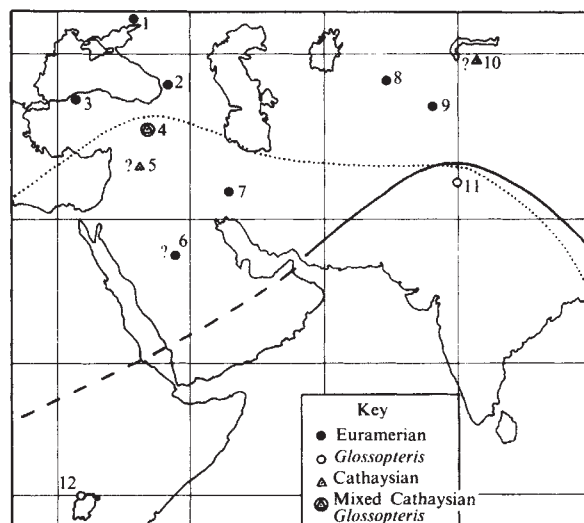


Fig. 1 Fossil plant occurrences relevant to the Unayzah flora, of Carboniferous to Permian age. 1, Donbass, USSR (Carboniferous)¹⁰; 2, Caucasus Mts (Carboniferous)¹⁰; 3, Zonguldak, Turkey (Carboniferous)¹⁰; 4, Hazro, Turkey (late Permian)¹³; 5, Ga'ara, Iraq (late Permian)¹²; 6, Unayzah, Saudi Arabia (late Carboniferous-early Permian); 7, Chal-i-Sheh, Iran (Carboniferous)⁴; 8, Uzbekistan, USSR (Carboniferous)¹⁰; 9, Fergana region, USSR (Carboniferous and early Permian)¹⁰; 10, Malajsary, USSR (Permian)¹⁰; 11, Kashmir (Carboniferous and early Permian)¹⁰; 12, Entebbe, Uganda (Permo-Carboniferous)¹¹. The two boundary lines indicate the northernmost limits of the *Glossopteris* flora according to Wagner¹³ (.....) and W.G.C. and Lacey¹¹ (---- and —). The question marks beside localities 5, 6 and 10 relate in each case to uncertainty in the assignment to the floral province indicated by the symbol.

Saudi Arabia has hitherto represented a blank area on palaeogeographic maps of Palaeozoic floras^{10,11}. The nearest records of more or less contemporaneous floras are those of Iraq¹² (late Permian, Fig. 1:5), Iran⁴ (Carboniferous, Fig. 1:7) and Turkey¹³ (late Permian, Fig. 1:4) to the north, the *Glossopteris* floras at Entebbe (Permo-Carboniferous, Fig. 1:12) 3,000 km to the south, and Kashmir (Permo-Carboniferous, Fig. 1:11) at a similar distance to the north-east. Palaeogeographic reconstructions of Permo-Carboniferous land masses based on palaeomagnetic evidence generally show the Arabian peninsula in juxtaposition with the African block, much as in its present configuration, but lying to the south of the Tethyan Gulf, and hence contiguous with the land mass occupied by the *Glossopteris* flora of Gondwanaland to the south and west. The Unayzah flora comes from a region which has hitherto been considered¹¹ to include the boundary of northern and Gondwana floras. The outlying record of *Glossopteris* at Hazro (Fig. 1:4) is made the basis for the northern boundary of the *Glossopteris* flora by Wagner¹³ (dotted line, Fig. 1), but W.G.C. and Lacey¹¹ query the validity of this record and show a more southerly position for this boundary (solid and broken lines, Fig. 1). The geographical significance of the Saudi Arabian flora therefore lies in the extent to which it shows clear affinity with the major floral provinces around it, and particularly in the

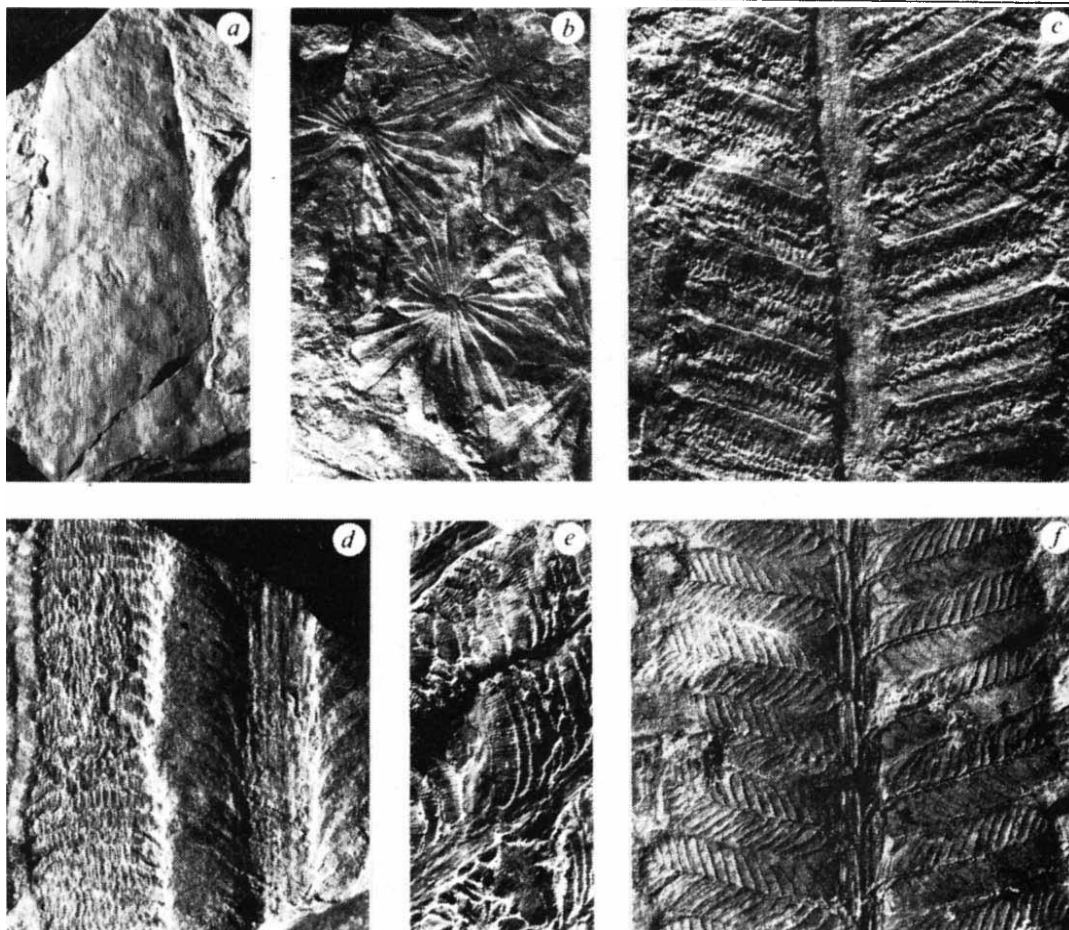


Fig. 2 Plant fossils from the base of the Khuff Formation, Unayzah. *a*, *Cordaites cf. principalis* (Germar), $\times 0.5$; *b*, *Annularia stellata* (Schlotheim) $\times 0.9$; *c*, Fertile *Pecopteris* $\times 4.7$; *d*, *Marattiopsis* sp. $\times 5.4$; pinna midrib at right, with intervening lamina between; *e*, the same, showing details of synangia on two adjacent pinnae, $\times 2.2$; *f*, *Pecopteris cf. hemiteloides* (Brgt.) Stur., $\times 3.1$. These specimens are deposited in the Geology Department of Riyadh University.

extent to which it may help clarify the northern boundary of the Gondwana province. Based on the present flora, it is clear that the most abundant and securely determined genera *Annularia*, *Pecopteris* and *Cordaites* are widespread throughout the Euramerian and Cathaysian provinces, whereas the *Marattiopsis*, if correctly determined, is known from more or less contemporaneous rocks only in eastern USSR. Apart from this taxon, the assemblage would conform with those of the Euramerian province within this period of time. In relation to the neighbouring floras of comparable diversity and age in Iraq¹² (Fig. 1:5) and at Hazro in Turkey¹³ (Fig. 1:4), the lack of genera characteristic of Gondwana and Cathaysian floras is significant because the neighbouring, although probably younger, Permian floras seem to contain these elements, but occur well to the north of Unayzah. Taken at face value, the plants from Unayzah would otherwise suggest that the northern boundary of the Gondwana flora lies somewhere to the south of the central part of the Arabian peninsula at that time.

Cytotoxic T-cell response to H-Y in 'non-responder' CBA mice

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Murine cytotoxic T-cell (T_c cell) responses to various antigens are controlled by immune response genes (Ir) mapping in the major histocompatibility complex (H-2). Both helper T cells, controlled by I region-coded genes, and T_c cells, controlled by K/D antigens, are necessary for a positive response. An H-2-restricted T_c -cell response to the male specific minor transplantation antigen (H-Y) can be elicited in B10 (H-2^b) female mice primed with syngeneic male spleen cells intraperitoneally (i.p.) or intravenously (i.v.), or by skin grafting followed by restimulation *in vitro* in mixed lymphocyte culture (MLR) with male cells¹. CBA (H-2^k) mice do not respond by these routes of *in vivo* priming, and this was thought to be due to a lack of permissible Ir genes for helper function². However, we now report that subcutaneous hind-footpad (fp) immunisation of 'non-responder' CBA mice with syngeneic male cells changes them to responders, a result which argues against a generalised Ir gene-controlled helper defect.

We tested B10 female and (CBA $\times$ B10) F_1 ($k \times b$) female spleen cells for their ability to mount an H-2-restricted T_c -cell response to H-Y *in vitro* after priming with male cells *in vivo*. B10 females were primed with 10^7 – 2×10^7 non-irradiated or irradiated male spleen cells, administered i.v. or i.p.; ($k \times b$) female mice were primed with either 2×10^7 CBA male or

Received 18 January; accepted 4 March 1980.

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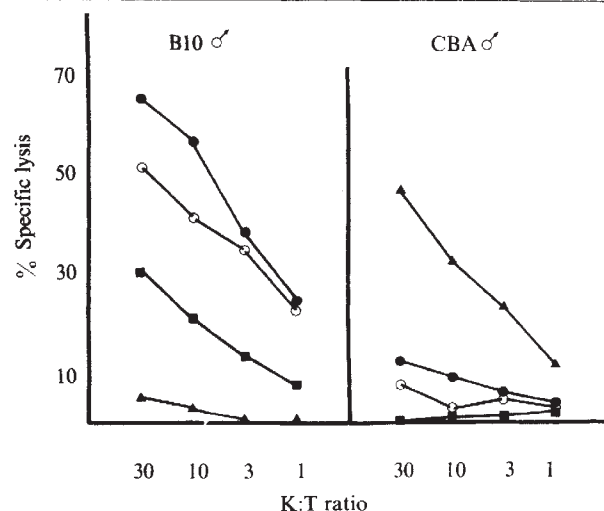


Fig. 1 Cytotoxicity of B10 and (k x b)_{F1} H-Y immune T_c cells. B10 female mice were given $1-2 \times 10^7$ unirradiated B10 male cells i.v. (●) or i.p. (○). (k x b)_{F1} mice were given either 2×10^7 2,000R irradiated CBA (▲) or B10 (■) male spleen cells i.v. Splenocytes of individual animals were removed at least 2 weeks after immunisation and cell suspensions prepared in RPMI supplemented with 10% heat-inactivated fetal calf serum (FCS), 2×10^{-5} M 2-mercaptoethanol and penicillin/streptomycin, and cultured at 2×10^6 cells ml⁻¹ for 5 d at 37 °C and 10% CO₂ with equal numbers of 2,000R-irradiated male stimulator cells homologous to the immunising cells. Thereafter, graded numbers of spleen cells were tested for cytotoxic activity against 2×10^4 ⁵¹Cr-labelled concanavalin A (Con A)-stimulated B10 male and CBA male lymphoblasts. (Spleen cells cultured for 24 h in RPMI supplemented with 10% FCS plus Con A at a concentration of $4 \mu\text{g ml}^{-1}$, cell concentration 5×10^6 per ml.) The cytotoxicity assay was carried out in triplicate in microtitre plates over a 3-h period with s.e.m. < 3%. The range of spontaneous ⁵¹Cr release was 8–20%. % Specific lysis was calculated according to the formula: % specific lysis = (experimental release – medium release / maximum release – medium release) × 100. K:T ratio, ratio of killer to target cells.

2×10^7 B10 male spleen cells irradiated with 2,000R to avoid a possible allogeneic effect and boosted *in vitro* with the same antigen as used for priming. As can be seen in Fig. 1, (k x b) H-Y immune T_c cells can lyse B10 male targets specifically when primed and boosted with B10 male cells and lyse CBA male targets when primed and boosted with CBA male spleen cells; these results show that H-2^k plus H-Y can be recognised by T_c cells in an immunogenic fashion, a result also shown by others^{1,3}. No differences could be detected in the B10 response when priming was carried out by different routes of immunisation, i.v. or i.p.

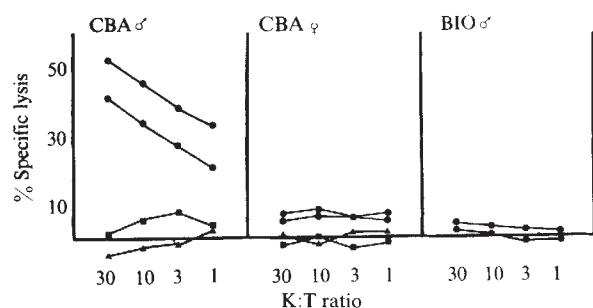


Fig. 2 Cytotoxicity of CBA H-Y immune T_c cells. CBA female mice were given $1-2 \times 10^7$ syngeneic non-irradiated male spleen cells i.v. (■), i.p. (▲) or fp (●). Spleen cells were boosted *in vitro* 5 weeks after priming, and assayed for cytotoxicity on CBA female, CBA male and B10 male targets as described in Fig. 1 legend.

The evidence for H-2^k plus H-Y association led us to investigate the possibility that H-2^k female mice can respond to H-Y when primed with CBA male cells. The two approaches taken were various doses of cells and different routes of administering cells. The results of the latter studies are shown in Fig. 2. CBA female mice primed i.p. or i.v. with syngeneic male spleen cells could not be boosted *in vitro* to generate an anti-H-Y response. This occurred over a dose range of 10^5-10^8 male spleen cells per mouse for priming and is in contrast to the results obtained with B10 and (k x b) mice. On the other hand, fp inoculation of $1 \times 10^7-2 \times 10^7$ CBA non-irradiated male spleen cells into CBA female mice resulted in the generation of a secondary *in vitro* T_c-cell response of equal strength to that obtained with B10 mice after i.v. or i.p. priming. The response is H-2 restricted and H-Y specific.

From the large number of CBA mice tested which gave a good H-Y-specific response, only one also caused significant cross-reactive lysis of male and female B10 targets. It is not known whether this reflects true cross-reactivity by one population of T_c cells or whether different populations are responsible for the result.

Neither spleen cells of CBA female mice primed with (k x b)_{F1} male cells i.v. (to generate possible help *in vivo* through an allogeneic effect) nor spleen cells from lethally irradiated CBA male mice reconstituted with CBA female spleen cells for more

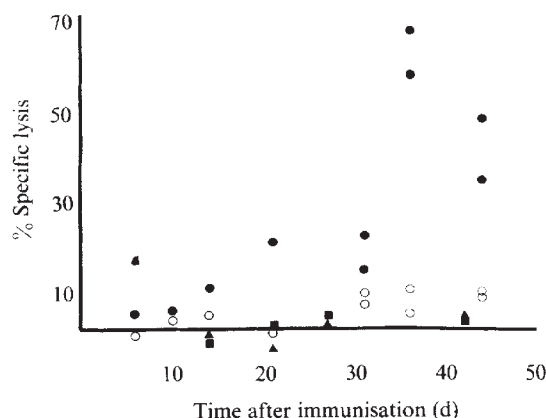


Fig. 3 Kinetics of anti-H-Y response in CBA mice. CBA mice primed as described in Fig. 2 legend were boosted *in vitro* 6–44 d post-priming and tested for cytotoxic activity on male (closed symbols) and female (open symbols) CBA lymphoblast targets. Values of 10:1 killer to target ratios from titration curves were used (details as in Fig. 1 legend).

than 2 weeks, could be boosted *in vitro* to generate an H-Y-specific T_c-cell response. Thus, only fp immunisation could generate a memory T_c-cell population specific for H-Y in H-2^k mice. A further difference between the response of B10 mice and CBA mice to the H-Y antigen lies in the time of appearance of the 'memory' T_c-cell pool in the spleens of primed animals.

Optimal or near-optimal responses in B10 can be obtained 2 weeks after priming, whereas with CBA mice, optimal results are only obtained 4–5 weeks after fp priming, although significant lysis is apparent after 2 weeks (Fig. 3). No responses were obtained at any time following i.v. or i.p. priming of CBA mice.

Our studies thus demonstrated that CBA mice are responders to H-Y antigen if they are immunised through the footpad and tested more than 4 weeks after immunisation.

These results are at variance with the interpretation of data obtained with H-2^k mice in chimaeric studies^{2,4,5}, in which it was suggested that H-2^k mice genetically lack the ability to generate H-Y-specific helper cells necessary for a T_c-cell response to H-Y, due to non-permissive Ir helper genes. This is in contrast to H-2^b mice, which apparently possess a permissive Ir helper gene.

As we have shown that mice of H-2^k haplotype can respond clearly to H-Y with a T_H-cell response, it must be postulated that T-helper cells are not necessary for the generation of a T_H-cell response to H-Y, at least, not after fp immunisation, or that i.v. and i.p. immunisation provides inadequate antigen presentation or induces a suppressor mechanism in H-2^k mice. We consider a lack of helper cell requirement unlikely, as helper T cells have been demonstrated both in H-2-restricted responses to viral antigens⁶ and in alloresponses⁷. Evidence for a suppressor mechanism in the antibody response to hen egg lysozyme has recently been shown, where a B10 non-responder after i.p. immunisation becomes a responder after fp immunisation⁸. Footpad immunisation may be physiologically a more natural route of priming and may be a very effective mechanism of antigen presentation, involving lymph node T-cell subsets, such as the T-initiator cells described by Cohen and Livnat⁹ capable of recruiting T_H-cell precursors or helper precursors. The difference in kinetics of the CBA fp response to the (k × b) i.v. response suggests a different mechanism is involved in eliciting a T_H-cell response to H-2^k plus H-Y.

We thank Dr W. Davidson and Professor N. A. Mitchison for reading the manuscript and A. Franklin for secretarial assistance.

Received 14 December 1979; accepted 18 February 1980.

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Expression of human α -globin genes in hybrid mouse erythroleukaemia cells depends on differentiated state of human donor cell

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We have developed a system which can be used to study the mechanisms that may govern the expression of human α -globin genes in human erythroid and non-erythroid haematopoietic cells^{1,2}. Human chromosome 16, which has been shown to bear the human α -globin genes³, is introduced by cell fusion into mouse erythroleukaemia (MEL) cells to generate continuously proliferating cell lines that retain permanently the human α -globin genes. We have shown that hybrid diploid MEL cells with human α -globin genes from erythroid donor cells express these genes fully through globin chain synthesis, while hybrid diploid MEL cells containing human α -globin genes from non-erythroid human haematopoietic donor cells contain very low levels of human α -globin mRNA and no detectable human α -globin chains. The levels of human α -globin mRNA in these hybrid cells were found to depend on factors present in the MEL recipient cell as well as on the differentiated state of the human donor cell, suggesting that this system may be suitable for characterisation of mechanisms governing haematopoietic differentiation in man.

We used normal fresh human haematopoietic cells as donor cells rather than established or transformed cell lines of fibroblasts or leukaemia cells. The Friend MEL cell was chosen as the recipient cell because it is committed to the expression of mouse globin genes; incubation of these cells in dimethyl sulphoxide (DMSO) increases cytoplasmic levels of mouse globin mRNA 50-100-fold (refs 4-6).

To ensure that the expression of the human globin genes in the hybrid MEL cells reflected the differentiated state of the donor

cells, we introduced the human genes by cell fusion because this does not alter the position of the genes in the donor genome, and the potential for alterations in the structure of the genes can therefore be minimised.

Hybrid cells derived by fusion of MEL cells and human haematopoietic donor cells lose human chromosomes during continuous culture. The biased loss of human chromosomes coupled with selection, based on the marker adenine phosphoribosyl transferase (APRT), for human chromosome 16 (refs 7, 8) which contains the human α -globin gene in man³, facilitates the isolation of cloned hybrid MEL cells which differ only in the cellular origin of this human chromosome.

We have reported that hybrid MEL cells bearing human α -globin genes derived from erythroid cells (populations of human marrow cells dominated by erythroid cells) fully express those genes^{1,2}. We have also shown that four independently derived hybrid cell clones which retain a tetraploid complement of MEL chromosomes (~80 mouse chromosomes) as well as human α -globin genes on human chromosome 16 from non-erythroid human haematopoietic donor cells, contain detectable levels of human α -globin chains⁷. Because these latter hybrid cells contained human chromosome 16 in the absence of all other human chromosomes, these studies showed that the only human chromosome which must be present in hybrid MEL cells

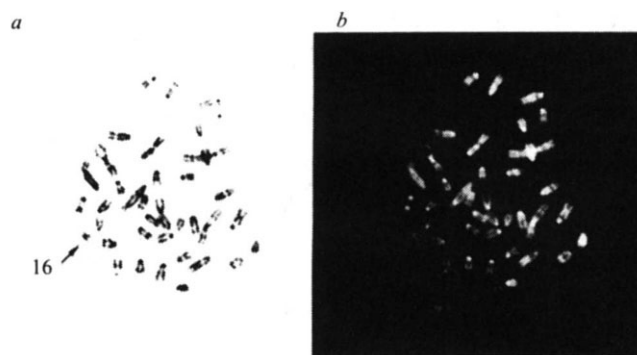


Fig. 1 Metaphase spread of hybrid cell derived by fusion of diploid MEL cells with non-erythroid human haematopoietic cells stained by Giemsa-trypsin banding (a) and Hoechst 33258 (b). Hybrid cells were isolated in alanosine-adenine medium which is lethal to unfused APRT-deficient MEL cells and promotes the growth of hybrid cells which have retained human chromosome 16^{2,7}. Human cells used for fusion were isolated from peripheral blood of donors by Ficoll-Hypaque centrifugation⁹. These cells contained less than 1/50,000 cells which stained positive for haemoglobin with benzidine⁷, and are known to contain less than 1/5,000 erythroid committed precursor cells¹⁰. Neither we⁷ nor others^{11,12} have detected human α -globin mRNA in such populations of mononuclear human cells or in leukaemia cell lines of human origin with markers of myeloid or lymphoid differentiation. Thus our population of donor cells had no detectable haemoglobin or human α -globin mRNA, and was dominated by at least a 3-4-log excess of non-erythroid human haematopoietic cells. Thirty-five metaphase spreads of hybrid diploid MEL cells with non-erythroid human α -globin genes were analysed by incubating log phase cells in colchicine, exposing successively to hypotonic and fixing media and spreading on washed glass slides as before^{3,7}. Such slides were then exposed to trypsin and Giemsa stain so as to generate banding patterns specific for each of the human and mouse chromosomes^{7,13,14}. Each of the 35 metaphase spreads was photographed, destained in fixative, and exposed to the fluorescent dye Hoechst 33258, which generates a uniform light fluorescence in human chromosomes, but an uneven fluorescent pattern in most mouse chromosomes, being very intense in the region of the centromere. An example of such a sequentially stained metaphase spread of hybrid cell no. 3 is shown here. Human chromosome 16 is indicated by the arrow. Although this hybrid MEL cell contains a near diploid complement of MEL chromosomes and human chromosome 16, it has lost most of the other human chromosomes, as is the case for most hybrid cells derived by fusion of MEL cells with human haematopoietic cells.

Table 1 Chromosomal constitution of hybrid MEL cells

Property measured	Hybrid cell no. 1 (human erythroid × diploid MEL)	Hybrid cell no. 2 (human non-erythroid × diploid MEL)	Hybrid cell no. 3 (human non-erythroid × diploid MEL)	Hybrid cell no. 4 (human non-erythroid × tetraploid MEL)
(A) No. mouse chromosomes	37	39	39	81
(B) Human chromosomes				
4	20	0	0	0
5	50	0	0	0
6	60	0	0	0
16	80	100	100	100
21	0	0	11	0
22	0	0	22	0
(expressed as % of metaphase spreads analysed)				
(C) poly (A+) cytoplasmic RNA				
Human α -globin cDNA	1,050	ND	3,000	3,999
(D) Relative level of human α globin mRNA	1/350	ND	1/1,000	1/1,333
(E) Relative level of mouse globin mRNA	1/175	ND	1/22	1/100
(F) Human α globin mRNA				
Mouse globin mRNA (ratio of previous 2 ratios)	0.5	ND	0.02	0.08
(G) Ratio of $\frac{\text{human } \alpha}{\text{mouse } \alpha}$ globin genes	1/2	1/2	1/2	1/4
(H) Human α globin mRNA				
Mouse globin mRNA (corrected to the ratio of $\frac{\text{human } \alpha}{\text{mouse } \alpha}$ globin genes found in a diploid hybrid MEL cell)	0.5	ND	0.02	0.16
(I) Human α globin chains	Present	Undetectable	Undetectable	Present

APRT, chromosomes and globin chains of hybrid populations no 1 and no. 4 were analysed as before^{2,7}. In all cases, chromosomal analysis is based on more than 25 metaphase spreads per cell line, analysed by a combination of banding and Hoechst 33258 staining as outlined in Fig. 1. All human chromosomes found are listed above. Hybrid populations no. 1 and no. 3 were uncloned, while no. 2 and no. 4 were clonal. APRT was assayed as before^{2,8}. Human α -globin mRNA was determined in induced hybrid MEL cells after 72 h of exposure to DMSO by RNA-cDNA hybridisation. Cytoplasmic extracts of those cells⁷ were fractionated on columns of oligo (dT) as before¹⁶. Human α -globin mRNA, obtained from the reticulocytes of a nonthalassemic donor was purified from human β -globin mRNA first by electrophoresis on 5% polyacrylamide gels in 100% formamide^{7,17}. Human globin cDNA was generated using the more rapidly migrating band (predominantly human α -globin mRNA) as a template for avian myeloblastosis reverse transcriptase as before⁷. Preparative hybridisation was used as before⁷ to remove the 8–10% of this human α -globin cDNA that was complementary to non- α -globin mRNA. The human α -globin cDNA used was greater than 99% pure, and had a specific activity of 42,000 d.p.m. ng⁻¹. It migrated on 5% polyacrylamide gels as a single peak with a molecular weight of 230,000. Hybridisation was carried out as before^{2,7} in 50% formamide and 0.5 M NaCl at 66°C for 90 h; each 2- μ l reaction mixture contained 0.1 ng of cDNA in addition to the RNA from the hybrid cell. Hybrids were identified by resistance to S₁ nuclease^{2,17}. Because the hybrid cells contained no non- α -globin genes and the hybridisation conditions prevented cross hybridisation of mouse globin mRNA with human globin cDNA (Fig. 3, ref. 7), the percentage hybridisation of the human α -globin cDNA was a measure of the amount of human α -globin mRNA present. To obtain the data shown in row H of Table 1, we added various amounts of poly (A+) RNA from hybrid cells or human reticulocytes to a fixed amount (0.1 ng) of human α -globin cDNA and incubated in 2- μ l mixtures as described above. The percentage of human α -globin cDNA protected in each case was plotted against the ratio of poly (A+) RNA/human α -globin cDNA. The lowest ratio at which maximal protection of human α -globin cDNA was observed is given in row C. We then divided the ratios for each cell type given in row C into that for human reticulocytes (which was determined to be 3 in the same experiments), to generate an estimate of the relative level of human α -globin mRNA in each hybrid cell (shown in row D). We then conducted the same analysis using mouse reticulocytes and mouse globin cDNA to estimate the relative level of mouse mRNA in each hybrid cell, the results of which are shown in row E. The final levels of mouse globin mRNA in MEL cells vary as a function of induction and are not affected by the presence or absence of human chromosome 16 from either erythroid or non-erythroid donor cells; similar amounts of mouse α and β -globin chains were present in both these hybrid populations. We then divided the human α -globin mRNA levels (row D) by the mouse globin mRNA levels (row E) for each hybrid cell to normalise the human α -globin mRNA levels for the induction process variables. These normalised estimates of human α -globin mRNA generated for hybrid cells with human α -globin genes derived from donor cells of different types (given in row F) can then be compared to determine if the potential for human α -globin gene expression varies in different types of donor cells. These ratios following correction for the ratio of human α /mouse α -globin genes found in a hybrid MEL cell with a diploid complement of mouse chromosomes and a single human chromosome 16, are given in row H. ND, not done.

for full expression of human α -globin genes is chromosome 16.

In the study reported here, we isolated two different populations of hybrid cells (Table 1) derived by fusion of diploid APRT-deficient MEL cells with non-erythroid human haematopoietic cells in conditions outlined in Fig. 1. Neither human donor exhibited any abnormalities of globin gene expression in peripheral blood or marrow erythroid cells. Analysis of these hybrids by staining of metaphase spreads showed that an intact human chromosome 16 was retained in more than 80% of the cells studied while most of the other human chromosomes had been lost (as shown in Table 1 and Fig. 1). The presence of human APRT in these hybrid cells also confirmed the presence of human chromosome 16 (Table 1). As Fig. 2 and Table 1 (row I) show, there were no detectable human α -globin chains in either of the diploid MEL hybrid cells containing non-erythroid human α -globin genes, in spite of the presence of normal levels of mouse α - and β -globin chains.

Table 1 shows that the cloned hybrid MEL population no. 2 (devoid of human α -globin chains) and no. 4 (positive for human α -globin chains), both of which retained human α -globin genes from non-erythroid haematopoietic cells, differed only with respect to the presence of a diploid (no. 2) or a tetraploid (no. 4) complement of MEL chromosomes. We therefore compared the levels of human α -globin mRNA in these cells, by the methods outlined in Table 1. The relative level of human α -globin mRNA in the hybrid tetraploid MEL cells (no. 4) which contain non-erythroid human α -globin genes was 0.16 (Table 1, row H), eight times higher than that found in diploid MEL cells, suggesting that the MEL cell contained some factor or process which depended on the dosage of MEL chromosomes in the hybrid cell, and which resulted in higher steady state levels of human α -globin mRNA.

We next compared the relative levels of human α -globin mRNA in the DMSO-induced hybrid MEL cells no. 1 and no. 3,

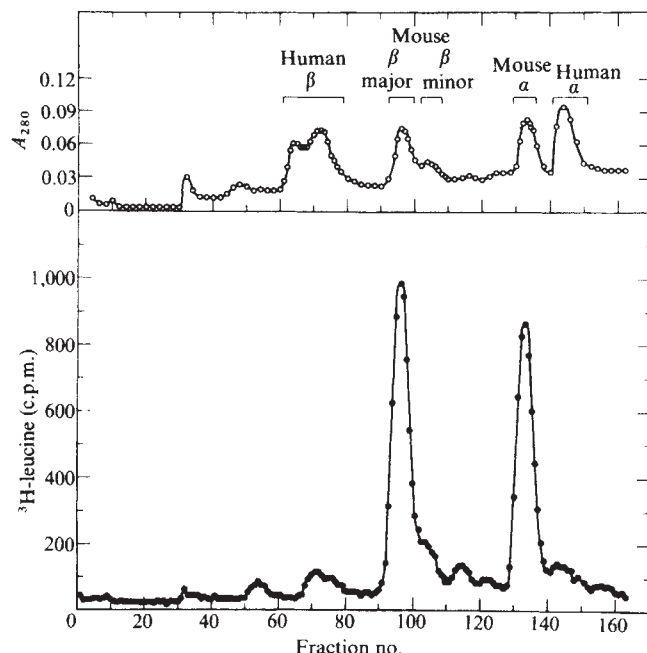


Fig. 2 Pattern of globin chain synthesis in hybrid cells derived by fusion of non-erythroid human haematopoietic cells with diploid MEL cells. 5×10^6 cells from the hybrid cell population were collected after incubation for 5 d in 1.25% DMSO. Cells were then incubated for 6 h in 0.5 mCi of ^3H -leucine specific activity 50 mCi mmol^{-1} dissolved in 25 ml of Ham's F-12 medium supplemented with 10% fetal calf serum. Fractionation of haemoglobin from other labelled cytoplasmic proteins was carried out on a 2-ml bed volume carboxymethyl cellulose column in 0.01 M phosphate buffer as discussed previously^{4,7}. After extraction of haem, globin chains were fractionated in 8M urea–0.01 M phosphate at pH 6.9 on columns of carboxymethyl cellulose¹⁵. The solid circles define the elution profile of ^3H -leucine-globin chains from hybrid cell population no. 3, using a 2-ml fraction volume. Open circles define the elution profile of unlabelled globin chains added as markers.

both of which contained a diploid complement of MEL chromosomes as well as human α -globin genes from erythroid and non-erythroid haematopoietic cells respectively (Table 1). The ratio of human α -globin mRNA to mouse globin mRNA in hybrid population no. 1, the hybrid MEL cell which contains a single human chromosome 16 derived from erythroid donor cells, is 0.5, as shown in Table 1, row F. This ratio corresponds to an average of 3,000 copies of human α -globin mRNA per cell. In contrast, in the hybrid diploid MEL cell no. 3 which contained human α -globin genes on human chromosome 16 from non-erythroid haematopoietic cells, this ratio was only 0.02 (Table 1, row F), which is one-twenty-fifth (120 copies of human α -globin mRNA per cell) of that found when human α -globin genes come from an erythroid donor cell. Because hybrid no. 3 contains human chromosome 16 in the absence of all other human chromosomes, the data suggest that the mechanism which mediates the decreased potential of human α -globin genes in non-erythroid human haematopoietic donor cells is *cis*-effective and the pattern of expression of human α -globin genes in the hybrid diploid MEL cells is dependent on the differentiated state of the human donor. Whether these differences in human α -globin gene expression arise from differences in the DNA itself or from some other fixed chromosomal state which stabilises the expression of human α -globin genes cannot be concluded. The data suggest that at least one mechanism limiting expression of human α -globin genes in non-erythroid haematopoietic cells is *cis*-effective. Further studies in this system of the pattern of expression of human α -globin genes in mouse erythroleukaemia cells may help to clarify which regions of the gene or its neighbouring sequences are important in the regulation of globin gene expression in differentiated and undifferentiated cells.

We thank Sylvon Von Der Pool and Mary Blenkush for technical assistance. Avian myeloblastosis reverse transcriptase was obtained through the Program Resources and Logistics Branch, Viral Oncology Program, National Cancer Institute. Dr A. Nienhuis donated RNA from the reticulocytes of a patient with classical deletion haemoglobin H disease. We also thank Drs Sydney Brenner, George Stamatoyannopoulos, Robert Church, Arthur Nienhuis and Dante Picciano for reviewing the manuscript.

Received 9 November 1979; accepted 5 February 1980.

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Molecular consequences of deletion formation mediated by the transposon Tn9

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Insertion elements and transposons stimulate deletion formation, mediating the elimination of neighbouring host sequences at a high rate compared to the background of deletions occurring in their absence. The (non-lethal) deletions generated by a variety of transposable elements end at or near the terminus of the element¹ (for reviews see refs 2–4). The finding that transposable elements generate the repetition of a short segment of host DNA on either side of the integrated element^{5,6} raises new questions about deletion formation at the sequence level. Do the deletions terminate at the precise endpoint of the element and remove the repeated sequence, or do they end within it or leave it intact? Is deletion formation linked to the transposition process? To study the above questions we have used the transposon Tn9, which carries the genetic determinants for chloramphenicol resistance flanked on either side by the insertion sequence IS1^{7,8} (in the same orientation), and which like IS1 generates a nine base pair repeat upon integration^{5,6,9}. Starting with a Tn9 integrated into the *lacI* gene of *Escherichia coli*, we examined deletions extending into the neighbouring *lacZ* and *lacY* genes. Sequence analysis, reported below, shows that after deletion formation the ends of the transposon remain intact, although one copy of the repeated 9 base pairs is removed by the deletion, fusing the end of Tn9 to host sequences several thousand base pairs away (in these cases). (Studies with Tn3¹⁰, IS1¹¹ and IS2¹² are consistent with these results.) We also find that the Tn9-distal end of the deletions terminate preferentially within the same region of DNA in which Tn9 insertion points cluster, suggesting that the processes of deletion formation and transposition share a common pathway.

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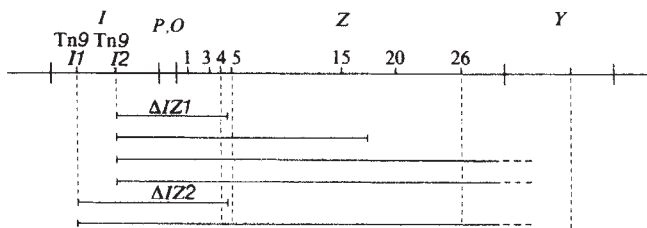


Fig. 1 The map position of several Tn9 mediated deletions is shown. The starting strain, X7733, used for the generation of deletions has been described previously²³. It carries Tn9 in the *I* gene of an *F'* *lacproB* episome and a *galE*⁻ mutation and a *lacproB* deletion on the chromosome. Although deletions can extend in either direction, only those causing the Z⁻ phenotype could be scored easily. The insertions *lacI1::Tn9* and *lacI2::Tn9* were used⁹. The map position of several Tn9-mediated deletions is shown. Cultures from single colonies were grown at 34 °C and streaked onto glucose minimal plates containing phenyl-β-D-galactoside²⁴ and the indicator dye 5-bromo-4-chloro-3-indoxyl-β-D-galactoside (Xgal)²⁵. The starting strain cannot grow on this medium, although Z⁻ survivors form white colonies. From each culture four to eight colonies were picked and the Z⁻ mutations mapped against the Y⁻ point mutation YA7047 contained in strain CSH7²⁵. Z⁻ colonies were present between 10⁻³ and 10⁻⁴ of the population, and ~15% of these gave Lac⁺ recombinants with CSH7. The respective mutations were mapped against other point mutations in Z (CSH1-6, CSH8-11)²⁵. Only one deletion mutation derived from the same original culture was analysed. The Z⁻ point mutations shown in the above figure are in one of 27 intervals in the gene²⁵, indicated by the figures above the line. The deletion mutations ΔIZ1 and ΔIZ2 were chosen for subsequent sequence analysis.

The starting strains for this work carried a Tn9 insertion in the *lacI* gene of an *F'* *lacproB* episome (see legend to Fig. 1). Deletions can extend in either direction from the integrated element, although only those deletions which prevent Z expression can be easily detected in this system. We examined independently occurring Z⁻ derivatives from two different *lacI::Tn9* insertions and mapped them against different point mutations in the *lac* region. Figure 1 shows the map position of several deletions detected in this manner.

The two deletions *lacI1::Tn9*ΔIZ1 and *lacI2::Tn9*ΔIZ2 were crossed genetically onto a plasmid derived from pMB9, pMC4⁵, which contains *lacI* and most of *lacZ*, using techniques described previously^{5,9}. Figure 2 depicts the restriction mapping and sequencing of the deletion endpoints. In each case, while the left juncture of Tn9 in the *I* gene is intact, the right end of Tn9 is now fused to sequences in the interior of Z. The element is now flanked by unrelated sequences of nine base pairs (Fig. 3). The exact ends of Tn9 are preserved. This includes 10 base pairs of the right arm and 50 base pairs of the left arm (as drawn in Figs 2 and 3) for Tn9 in ΔIZ2, and 65 base pairs of the right arm of Tn9 in ΔIZ1. Moreover, the *Hae*III restriction pattern is indistinguishable from the starting Tn9 in both cases.

As Fig. 3 shows, the derivatives of Tn9 resulting from deletions extending into the Z gene are no longer flanked by repeated sequences of nine base pairs. We determined the frequency of transposition of these Tn9 derivatives, as well as the starting Tn9 elements. Tables 1 and 2 show that the frequency of transposition is unaffected by the removal of the nine base pair repeat.

Table 1 Coincidence formation mediated by Tn9 derivatives

pMC4 donor plasmid	Frequency of Cam ^r colonies
<i>lacI2::Tn9</i>	$4.7 \pm 0.7 \times 10^{-6}$
<i>lacI2::Tn9</i> ΔIZ2	$4.5 \pm 0.5 \times 10^{-6}$

An F factor carrying a non-transposable *kan*^r marker (courtesy of Dr F. Heffron) was used to detect transposition of Tn9 from the pMC4 plasmid. The donor strain (R90C) background was *recAΔ(lacproB) rif^rnalA*. The F⁺ factor was transferred to a Str^r recipient (Q90C) and the approximate frequency of transposition calculated by monitoring the proportion of Cam^rKan^rStr^r colonies among the Kan^rStr^r colonies, averaged for 20 experiments in each case. (The error given in the Table represents the standard deviation.) Most of the colonies are Tet^r, since the plasmid was transferred due to a presumed coinfectant intermediate in the transposition process, although 0.2% of the colonies were Tet^r in each case. That the transfer of the plasmid is mediated by Tn9 was shown by the fact that almost no transfer (1.5×10^{-8}) of Tet^r is found when the pMC4 plasmid does not contain Tn9.

We examined the distribution of deletion endpoints in the *lac* region by characterising independently-occurring deletions generated by *lacI1::Tn9*. Only the Z⁻ phenotype was selected for initially, so any deletion extending into or past the Z promoter could be detected. We tested the *lacZ* mutations for recombination with the late Y⁻ amber mutation MAB19¹³. We subsequently mapped 65 of these deletions against a series of mutations in the Z gene and the Y gene. As Fig. 4 shows, most of the deletions (~80%) end within a region consisting of the very end of the Z gene and most of the Y gene, even though this region is only about one third of the size of the Z gene (assuming that Z and Y are not separated by a very large region). However, the deletion endpoints are distributed in many different places both in Z and Y.

We have examined the sequence alteration produced by two different Tn9-mediated deletions extending from the *lacI* gene into the *lacZ* gene. In both cases the deletions end at the exact

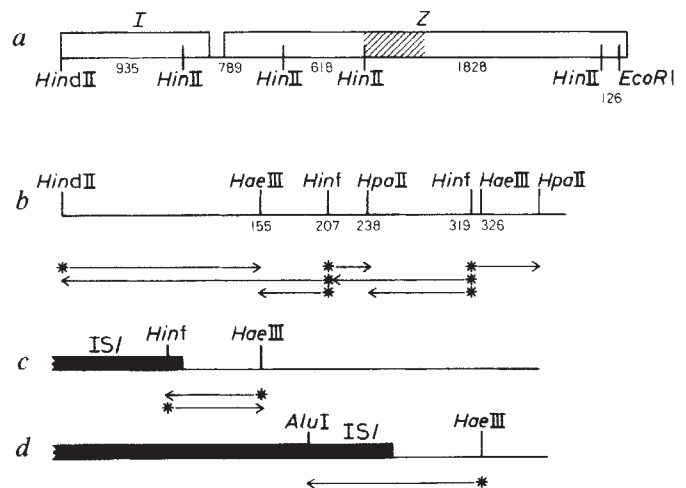


Fig. 2 Restriction mapping and sequencing deletion endpoints. *a*, The HindII cuts in the *lacI* and Z genes. Distance between cuts is given in base pairs (bp). The single R1 cut in the region occurs 126 bp beyond the final *Hin* cut and 53 bp from the end of Z. The two Tn9-mediated deletions were crossed to pMC4, which contains this region up to the R1 cut. In a plasmid containing Tn9 in *I*, the 935 bp *Hin* fragment is replaced by a fragment 3,600 bp long (the 2,700 bp Tn9 has no *Hin* cuts). In plasmids carrying the deletions, the corresponding band is now 4,600 bp and comprises the first part of the 935 bp fragment of *I*, Tn9 and most of the 1,828 bp fragment of Z. The 789 and 618 bp *Hin* fragments are missing. A comparison of the restriction map of the 4,600 bp *Hin* fragment with the restriction map obtained from the wild-type *Hin* 1,828 bp fragment indicates that both deletions end within the first few hundred bases of the 1,828 bp fragment. Therefore, this wild-type region, in the centre of the Z gene (indicated by shading), was sequenced. *b*, Restriction map of the first 400 bp of the *Hin* 1,828 bp fragment. Distances are given in bp from the *Hin* cut. Fragments sequenced are indicated by arrows. *, Indicates ³²P. Sequencing was done by the method of Maxam and Gilbert²⁶. Sequences on the upper strand were obtained by cutting a *Hin*-*Hpa* 238 bp fragment labelled with ³²P with *Hae*III, yielding the 155 bp fragment sequenced. A labelled *Hin*-*Hin* 112 bp fragment was recut with *Hpa* and the resulting 31 bp fragment was sequenced. A labelled *Hin*-*Hin* fragment approximately 800 bp long was recut with *Hpa* and a fragment approximately 70 bp long was sequenced. On the lower strand, labelled *Hin*-*Hin* 207 bp and *Hin*-*Hin* 112 bp fragments were strand separated and sequenced. Also, the 207 bp fragment was recut with *Hae* and a 52 bp fragment was sequenced, and the 112 bp fragment was recut with *Hpa* and an 81 bp fragment was sequenced. *c*, Junction of Tn9 and Z in *lacI2::Tn9*ΔIZ2. Starting with the 4,600 bp *Hin* fragment, a 365 bp *Hae* fragment extending from 210 bp inside Tn9 (IS1) to the *Hae* cut 155 bp from the *Hin* cut was isolated, labelled with ³²P, and recut with *Hin*, yielding a 73 bp fragment which gave the juncture sequence on the lower strand. The upper strand of the same juncture was sequenced by labelling a 105 bp *Hin* fragment extending from 10 bp inside IS1 to the *Hin* cut 207 bp from the *Hin* cut, recutting with *Hae*, and sequencing 73 bp junction fragment. *d*, The sequence of the juncture of Tn9 and Z for *lacI1::Tn9*ΔIZ1 was obtained by isolating and labelling the 281 bp *Hae* fragment extending from the *Hae* cut 210 bp inside IS1 to the *Hae* cut 326 bp from the *Hin* cut. This fragment was recut with *Alu* and the 136 bp fragment giving the lower strand juncture was sequenced. For both deletions the left juncture (in the *I* gene) was shown to remain intact by restriction mapping. In *lacI2::Tn9*ΔIZ2 the left juncture was also sequenced, using the *Hae*-*Hpa* junction fragment described⁹ for *lacI2::Tn9* and is identical to the juncture sequence reported there.



Fig. 3 *a*, The sequence of 360 base pairs starting from the *Hind*III cut at the end of the 1,828 base pair fragment, as determined as shown in Fig. 2. Numbers refer to amino acids in the β -galactosidase sequence²⁷. There are two minor differences from the reported protein sequence at positions 356 (Gln to Glu) and 412 (Asp to Asn). They are indicated by *. The position of the deletion endpoints of *lacI*::Tn9Δ121 and *lacI2*::Tn9Δ122 are shown; only the last 10 base pairs of Tn9 and the first few bases to which it is fused are shown. *b*, The nine base pair sequences now flanking Tn9 in the two deletions. Each Tn9 insertion was flanked by a direct repeat of the sequence shown on the left. After deletion formation it is flanked by unrelated sequences of 9 base pairs.

terminus of the element, eliminating one copy of the nine base pairs of *I* gene DNA which were repeated during the integration of Tn9. This result extends the genetic and biochemical work of other authors, which indicated that such deletions end within 50 base pairs or less of the respective terminus of the element¹⁻⁴. Sequence studies with IS1, IS2 and Tn3-mediated deletions are also consistent with the results reported here¹⁰⁻¹².

Tn9 derivatives which are no longer flanked by repeated sequences of 9 base pairs transpose with normal frequencies (Tables 1 and 2), strengthening arguments that Tn9 transposition does not involve homologous pairing by this flanking sequence, but rather generates the repeated sequence during the process of insertion itself⁹. (Experiments with Tn10 also argue that the flanking repeated sequences are not necessary for transposition¹⁴.)

Genetic mapping of deletion endpoints in *Z* and *Y* shows that 80% of the endpoints fall within a region extending from the extreme distal end of *Z* to the end of *Y* (see Fig. 4), a region one third of the size of the remainder of *Z*; within this preferred region deletion endpoints occur at many different positions. Transpositions of Tn9 into *Z* and *Y* show a similar strong preference for *Y* and the end of *Z*, as ~98% of Tn9 transpositions into *Z* and *Y* occur in this region, and like the deletion endpoints the transpositions fall at many positions within it (ref. 9 and J.H.M., M.P.C. and D. Galas, unpublished results). Coincident specificities of both deletion endpoints and transposition integration points have also been reported for Tn3¹⁵, Tn1¹⁶ and Tn10¹⁷, and argue for a common step in the mechanism of both events.

Table 2 Transposition of Tn9 derivatives to *F*⁺*kan*

Tn9 derivative (on chromosome of CSH51)	Frequency of Cam ^r , Kan ^r colonies (R90C background)
<i>lacI</i> ::Tn9	3.8×10^{-5}
<i>lacI</i> ::Tn9Δ121	4.0×10^{-5}
<i>lacI2</i> ::Tn9	3.8×10^{-5}
<i>lacI2</i> ::Tn9Δ122	3.5×10^{-5}

The Tn9 insertions were crossed by genetic recombination into the *lac* region of strain CSH51²⁴, which harbours a *lacProB* deletion and carries a ϕ 80dlac prophage. (In order to determine that the recombinant carried the original insertion mutation, backcrosses were used, in which the insertion was re-crossed into an *F*⁺*lacProB* episome and subsequently mapped and characterised.) After introduction of an *F*⁺*kan* factor these strains were used as donors in a cross against R90C (see legend to Table 1). The frequency of Cam^r, Kan^r, Rif^r colonies among the Kan^r, Rif^r colonies was determined in several experiments. The Cam^r, Kan^r colonies, when cured of the *kan*^r episome, also lost the Cam^r character, demonstrating that the Cam^r was carried by the *F*⁺ episome.

How does a transposable element cause a deletion in its vicinity? These sequence results show that, as in transposition, the exact end of the transposable element must be an active participant. Moreover, studies with bacteriophage Mu, a transposable element, show a structural requirement for both ends of Mu in deletion formation¹⁸; if one can generalise from this result then, presumably, both ends of Tn9 are involved in deletion formation. These findings and the coincident specificity of deletion endpoints and transposition integration sites indicate a close relationship between transposition and deletion formation. Models giving a specific mechanism linking deletion formation to transposition have been proposed^{19,20}. Shapiro²⁰ has outlined a mechanism for transposition in which a copy of the transposable element is replicated into the target site. When this mechanism is applied to transposition to a nearby site within the same replicon, in one orientation a deletion will result generating a fusion of the end of the transposable element to a new sequence, having the structure that we found, plus another copy of the transposable element fused to the deleted material. In the opposite orientation a second copy of the transposable element occurs in inverted orientation, along with inversion of the intervening material. (We have not examined inversions in our study of *Z*⁻ mutations.) Thus, a deletion may be the result of a transposition event, when the target for transposition is an adjacent region on the same chromosome. In this regard it would be interesting to examine the effect on deletion formation of host mutations affecting transposition frequencies, and vice

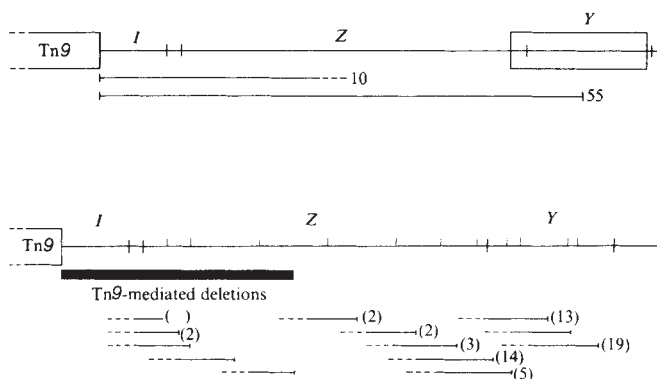


Fig. 4 The distribution of deletion endpoints generated by Tn9. The Tn9 insertion *lacI*::Tn9 was used to generate deletions in *Z* and *Y*. The top part of the figure shows that 10 of 65 deletions terminate within the major portion of *Z*, whereas 55 deletions end in *Y* or at the very end of *Z*. As can be seen in the lower part of the diagram, many different endpoints are involved. The deletions were mapped against point mutations in *Z*²⁵ and *Y*¹³ whose positions are indicated by the vertical lines along the lower part of the figure. The values in parentheses indicate the number of deletions with endpoints in this interval. Experimental details were the same as those given in the legend to Fig. 1, except that 32 °C was used, and the late *Y*⁻ mutation *MAB19*¹³ mapping at the far end of the *Y* gene was used for initial screening. Approximately 25% of the *Z*⁻ mutations were deletions ending before *MAB19*.

versa. One mutation that apparently decreases the rate of IS1-mediated deletions has been reported²¹.

The structure of *int*-mediated deletions from the *att* site of phage λ is analogous to the structure of deletions mediated by transposable elements and has been interpreted in a similar fashion; that is, as integration events into adjacent regions of the λ chromosome instead of into the normal bacterial *att* site²².

We thank Dr L. Johnsrud, who participated in the early stages of this work, Drs B. Müller-Hill, A. Hobson and F. Heffron for bacterial strains, Drs W. Gilbert, P. Nevers and H. Saedler for helpful discussions, and M. Hofer for technical assistance. This work was supported by a grant from the Swiss National Fund (3.493.0.79) (to J.H.M.) and by an NIGMS grant (to Dr W. Gilbert).

Received 20 September 1979; accepted 18 February 1980.

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Migration of capillary endothelial cells is stimulated by tumour-derived factors

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Angiogenesis, the growth of new blood vessels, occurs normally during osteogenesis, luteinisation and the development of the embryo, and in pathological states such as chronic inflammation, certain immune reactions and neoplasia¹. Furthermore, solid tumours have been reported to secrete a diffusible factor which promotes the directional growth of new capillaries towards a growing tumour². Two events required for the formation of a new capillary in response to an angiogenesis factor *in vivo* are the migration and subsequent proliferation of capillary endothelial cells³. Progress in purifying angiogenesis factors and studying their action has been hindered, however, by the lack of quantitative *in vitro* assays for capillary cell migration and proliferation. Recently, we have been able to isolate clonal cell lines of bovine capillary endothelial cells that can be maintained in long-term culture using tumour-conditioned growth medium⁴. I now report a quantitative *in vitro* assay for endothelial cell migration based on the phagokinetic track assay of Albrecht-Buehler⁵. The evidence presented here demonstrates that tumour-derived factors stimulate the migration of capillary endothelial cells whereas the same factors have no effect on the migration of aortic endothelial cells.

In the elegant system devised by Albrecht-Buehler to analyse the movement of cultured cells, the cells are first plated onto glass coverslips that have been previously coated with gold particles⁵. The cells ingest the gold and, as they move, leave bare areas or tracks that serve as a record of their movement. Figure 1 demonstrates the phagokinetic tracks made by bovine capillary endothelial (BCE) cells during an 18-h period. In normal growth medium containing 10% calf serum, little phagokinetic movement takes place (Fig. 1a). In contrast, when the assay is carried out in medium conditioned by mouse sarcoma 180 cells, phagokinetic movement increases and the size of the tracks is significantly larger than for cells incubated in non-conditioned medium (Fig. 1b).

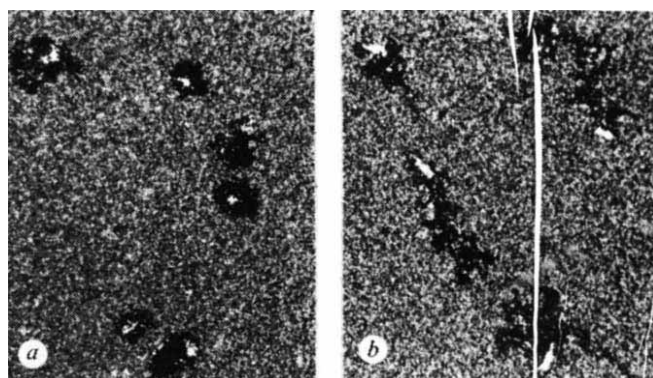


Fig. 1 Tumour-stimulated migration of bovine capillary endothelial cells. Bovine capillary endothelial cells (3,000) were seeded on gold-coated 22 × 22 mm glass coverslips prepared according to the method of Albrecht-Buehler. The cultures were incubated at 37 °C in Dulbecco's modified Eagle's medium containing 10% calf serum (DME-CS) until the cells had attached to the coverslips (~4 h). The coverslips were then transferred to 35-mm culture dishes containing either DME-CS alone (a) or mixed 1:1 with sarcoma-conditioned medium prepared as described in ref. 4 (b). After an additional 18 h, the medium was removed and replaced with 2.5 ml of 10% buffered formalin phosphate (Fisher Scientific) to terminate the experiment. The phagokinetic tracks were observed under incident light with a Nikon MS inverted microscope and photographed using a Wild M Ka4 photoautomat camera. ×20.

It has been noted that an increased rate of cell movement will be reflected by an increased phagokinetic track size⁶, but there have previously been no reports of a quantitative migration assay based on this technique. In the experiments reported here, the precise dimensions of endothelial cell phagokinetic tracks were measured with the aid of a MOP-3 digital image analyser (Carl Zeiss) as described in Fig. 2 legend. For each experimental point, the outlines of 100 tracks were traced and analysed to determine the perimeter length, maximum diameter and area. Because an increase in cell locomotion does not always correlate with an increase in directionality of movement⁷, the pattern of the tracks is highly irregular and therefore an increase in phagokinetic movement can best be detected as an increase in the mean area of the tracks rather than by their absolute lengths. Therefore, in all figures, migration is expressed in units of area of the phagokinetic tracks (μm^2).

Quantitative measurement of BCE cell phagokinetic tracks reveals that there is a linear increase in track size as the concentration of tumour-conditioned medium is increased between 0 and 100% (Fig. 2). Whereas the capillary endothelial cells move slowly in the absence of tumour-conditioned medium and rapidly in its presence, aortic endothelial cells move rapidly regardless of the presence or absence of tumour-conditioned medium (Fig. 2). These results are consistent with our previously published observations on endothelial cell proliferation. Capillary endothelial cells grew slowly in normal growth medium but rapidly in tumour-conditioned medium whereas aortic endothelial cells grew rapidly in either⁴.

To determine whether medium conditioned by other cell

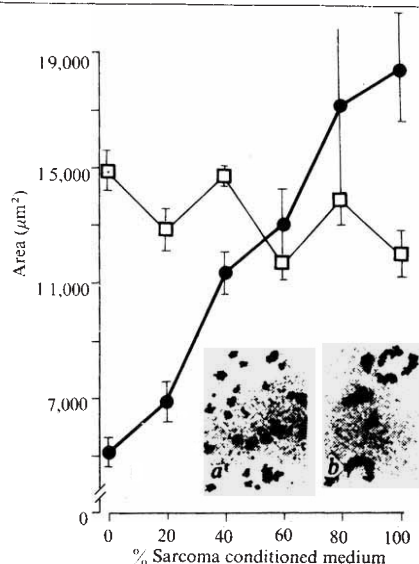


Fig. 2 Effect of sarcoma-conditioned medium on migration of bovine capillary and aortic endothelial cells. Bovine capillary endothelial cells (3,000) (●) or bovine aortic endothelial cells (□) were seeded on gold-coated coverslips as described in Fig. 1 legend and incubated for 18 h in DME-CS supplemented with the indicated concentration of sarcoma-conditioned medium. At the end of the experiment, the images of the phagokinetic tracks were transferred from the inverted microscope ($\times 4$ objective) to the screen of a Satchell-Carson 10M915 television by means of an RCA TC1005 video camera. The images were then traced from the television screen onto sheets of transparent plastic which were subsequently placed onto the magnetic tablet of a Zeiss MOP-3 digital image analyser. The tracings of the phagokinetic tracks were then retraced using the magnetic stylus of the MOP-3 and automatically processed for dimensions of area (shown above) as well as maximum diameter and length of perimeter. To eliminate consideration of cell division or cell collisions, only tracks formed by a single cell were analysed. Each point represents the mean area of 100 samples $\pm$ s.e.m. The inset shows the phagokinetic tracks of capillary (a) and aortic (b) endothelial cells in non-conditioned medium.

types might also stimulate endothelial cell migration, harvest fluid was collected from rapidly growing cultures of bovine aortic endothelial cells, bovine smooth muscle cells, BCE cells and mouse sarcoma 180 cells. When these media were tested for their ability to stimulate BCE cell phagokinetic movement (Fig. 3), the smooth muscle and aortic endothelial cell-conditioned media were completely inactive. Medium conditioned by the capillary endothelial cells themselves induced a 20% increase in the size of the phagokinetic tracks whereas the sarcoma cell-conditioned medium brought about a 90% increase. Once again, aortic endothelial cell tracks were large in the absence of any conditioning factors. None of the conditioned media caused an increase in the size of the aortic cell tracks to greater than 10% beyond control values (Fig. 3). In a second series of experiments, conditioned media from several other normal cell types had no effect on the migration of either capillary or aortic endothelial cells. The cell types tested included Swiss mouse 3T3 cells, F240 8 Fisher rat embryo fibroblasts, early-passage human

Table 1 Growth of capillary endothelial cells in medium conditioned by sarcoma 180 cells or aortic endothelial cells

Growth medium	Cell no. after 5 days
Non-conditioned medium (10% calf serum)	2.14×10^4
Sarcoma 180-conditioned medium	8.4×10^4
Aortic endothelial cell-conditioned medium	6.8×10^4

10^4 cells were seeded in gelatin-coated 16-mm tissue culture wells containing 1 ml of non-conditioned medium. After 18 h to allow plating, the medium was aspirated and replaced with test medium prepared as described in Fig. 3 legend. All test media contained a final concentration of 10% calf serum. Fresh test medium was added on day 3. On day 5, the cells were removed from the wells with 0.25% trypsin and counted with a Coulter Model Zf particle counter.

diploid skin fibroblasts, MDCK canine kidney epithelial cells and primary C57 mouse bladder epithelial cells. In contrast to these normal cells, medium conditioned by benzo(a)pyrene-transformed C57 mouse bladder epithelial cells had significant migration-stimulating activity (data not shown).

It could be argued that if migration were an obligatory response to any growth stimulus, the migration assay would simply be an indirect measure of mitogenic activity. However, aortic endothelial cell-conditioned medium stimulates the growth of BCE cells to a level nearly equivalent to the sarcoma conditioned medium (Table 1), but has no effect on the BCE cell migration as shown in Fig. 3. This indicates that BCE cell growth and migration are independent events and might therefore be mediated by separate factors.

In a preliminary attempt to determine whether a quantitative assay could be devised to detect angiogenesis factors based on their ability to stimulate endothelial cell movement, increasing concentrations of an extract of human hepatoma cells were tested for their ability to increase the size of BCE cell phagokinetic tracks. This same extract shows angiogenic activity in the rabbit cornea and the chick chorioallantoic membrane (data not shown). Figure 4 shows that an increase in track size can be detected with $0.1 \mu\text{g ml}^{-1}$ and reaches a peak at $100 \mu\text{g ml}^{-1}$. As neovascularisation is a complex phenomenon involving basement membrane hydrolysis, cell proliferation, cell junction formation and lumen formation in addition to cell migration³, it is unlikely that any single *in vitro* assay could totally replace the *in vivo* bioassays that have been developed to study angiogenesis¹. Nevertheless, the migration assay described here should provide a sensitive, rapid (18 h), quantitative assay for one critical component of angiogenesis.

The quantitative assay described above has already proved useful in demonstrating the effect of tumour-derived factors on

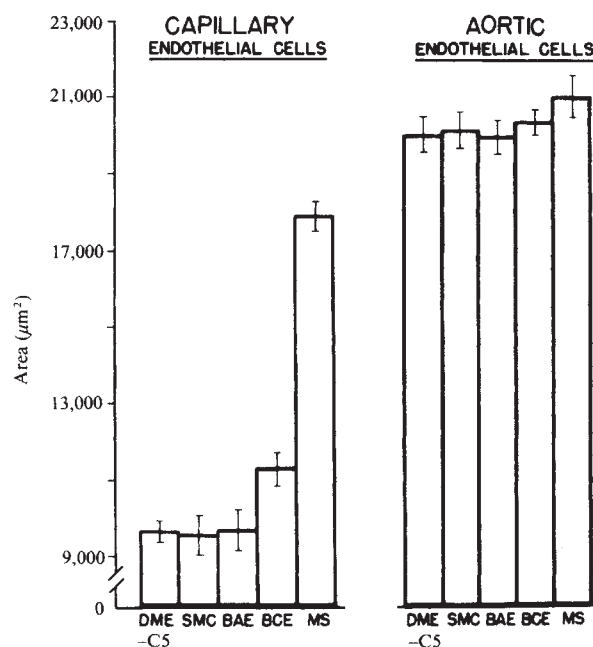


Fig. 3 Effect of conditioned medium from several different cell lines on the migration of bovine capillary and aortic endothelial cells. Bovine capillary endothelial cells (3,000) (left panel) or bovine aortic endothelial cells (right panel) were seeded on gold-coated coverslips as described in Fig. 1 legend and allowed to migrate for 18 h at 37°C in DME-CS that had been mixed 1:1 with conditioned medium prepared by 48 h incubation with sub-confluent cultures (6.6×10^4 cells cm^{-2}) of bovine aortic smooth muscle cells (SMC), bovine aortic endothelial cells (BAE), bovine capillary endothelial cells (BCE) and mouse sarcoma 180 cells (MS). For each sample, 100 phagokinetic tracks were traced from the television screen and retraced on the magnetic tablet of the Zeiss MOP-3 image analyser for determination of mean track area $\pm$ s.e.m.

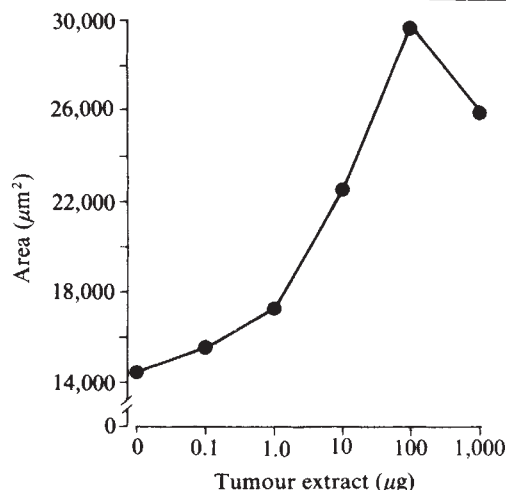


Fig. 4 Effect of human tumour extracts on capillary endothelial cell migration. Human hepatoma cell extracts provided by Dr B. Wildi were prepared by incubating suspensions of cultured hepatoma cells (5×10^7 cells ml $^{-1}$) in phosphate-buffered saline for 4 h at 4°C. The cells were removed by centrifugation and the resulting supernatant was concentrated and diluted in phosphate buffer (0.1 M NaH₂PO₄-0.02% NaN₃) four times to remove the saline. The final concentrate was applied to a CM-Sephadex C-50 column equilibrated with the 0.1 M phosphate buffer. The material that eluted with the starting buffer was collected, pooled, dialysed and lyophilised. Increasing concentrations of this material were added to 35-mm culture dishes containing 2 ml DME-CS. Gold-coated coverslips onto which 3,000 bovine capillary endothelial cells had been plated were transferred into these culture dishes and incubated for an additional 18 h at 37°C. Areas of the phagokinetic tracks were determined as described in Fig. 2 legend. Each point represents the mean area of 100 samples $\pm$ s.e.m.

capillary cell migration and in so doing has pointed out a fundamental difference between capillary endothelial cells and endothelial cells from larger vessels. Furthermore, it may lead to a new understanding of certain biological agents such as interferon, which we have recently found can act as a reversible inhibitor of cell migration⁸. The application of this method is not limited to endothelial cells. Phagokinesis can be studied with virtually any motile cell that adheres to a substratum^{5,6}. Thus, many cellular movements that were previously amenable to study only by time-lapse microcinematography may now be quantified by measurement of cell tracks. Possible uses could include the study of migration of neural cells, leukocytes and embryonic cells, the migration patterns of invasive and metastatic cells and the screening of cancer chemotherapeutic agents for anti-migratory activity.

I thank Drs F. Solomon, G. Albrecht-Buehler and J. Folkman for helpful discussions, and Ms C. Scheiner for technical assistance. The F2408 rat embryo fibroblasts were provided by Dr K. Steimer, the MDCK cells by Dr M. Klagsbrun, the normal and transformed mouse bladder epithelial cells by Dr Ian Summerhayes, and the human hepatoma cell extracts by Dr B. Wildi. This work was supported in part by a grant to Harvard University from Monsanto and by grant no. 1496-C1 from the American Cancer Society, Massachusetts Division.

Histone H1⁰ accumulates in growth-inhibited cultured cells

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The H1 class of histones contains several molecular species, even within a single organism¹. The combination of these subfractions varies according to the state of terminal differentiation^{2,3}, embryonic development⁴ and hormonal induction⁵. One of the subfractions, H1⁰, occurs at levels that are inversely correlated with the rate of cell division in mammalian tissues⁶. Although H1⁰ might be functionally or even structurally distinct from the usual H1 histone, Panyim and Chalkley⁶ established that its size and amino acid composition resembled other H1 histones. The quantitative studies of its relationship to mitotic index were greatly extended by Marks *et al.*⁷, and others⁸⁻¹² have measured H1⁰ contents during processes such as tissue regeneration and selective tissue destruction. All these observations were consistent with the notion that histone H1⁰ suppresses gene replication. One limitation in these demonstrations of the inverse correlation between H1⁰ and cell division is that there are many variable parameters, other than mitotic index, when comparing one tissue to another. This problem is largely overcome by comparing regenerating tissues to their normal counterparts⁸⁻¹¹. In the latter situation, it is possible that cell types which always have low H1⁰ contents are preferentially replaced early in the regeneration process, while different cell types with high H1⁰ levels build up in the tissue later. If it could be demonstrated that H1⁰ accumulated along with the arrest of cell division in a single cell type, these ambiguities would be removed. We report here such a demonstration using cultured cells.

Comparisons were made between the H1⁰ content of rapidly growing HeLa cells and that of cells whose growth had been inhibited by high cell density (after 10 days culture on a plate). Another comparison was made between H1⁰ levels in rapidly growing mouse neuroblastoma cells, and the same cells whose growth was inhibited by serum deprivation (4 days without serum). In both cases there were substantially higher amounts of H1⁰ (relative to other H1 histone) in the more slowly dividing cells. H1 histones were selectively extracted using perchloric acid (PCA) and submitted to polyacrylamide gel electrophoresis (Fig. 1). An electrophoretic band, assumed to be H1⁰ because of its mobility⁶, was clearly more pronounced in preparations from growth-inhibited HeLa cells, than in those from rapidly growing cells, and it was clearly present in higher amounts in extracts of serum-deprived neuroblastoma cells than it was in their rapidly

Table 1 Levels of histone H1⁰ in rapidly growing and growth-inhibited cells

Cell	Growth state	Relative H1 ⁰ content
Neuroblastoma	rapid growth	0.08 $\pm$ 0.01
	serum deprived	0.30 $\pm$ 0.06
HeLa	rapid growth	0.05 $\pm$ 0.01
	density inhibited	0.20 $\pm$ 0.02

Polyacrylamide gels were stained in aqueous 0.03% (w/v) Coomassie blue R-250, 24% (v/v) isopropanol, 10% (v/v) acetic acid; and destained in 0.003% Coomassie blue, 10% isopropanol, 10% acetic acid followed by washing in 10% acetic acid. The gels were scanned at 610 nm. H1⁰ content is given as the ratio of H1⁰ to the ordinary H1 which is essentially constant in all chromatin¹⁵. Each ratio is the average of 5 to 14 observations with the standard deviations shown.

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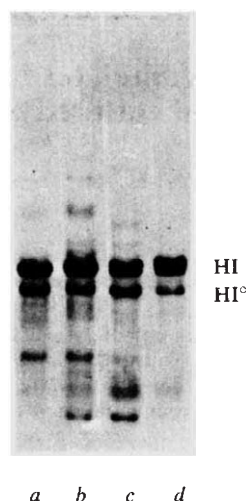


Fig. 1 Gel electrophoresis of perchloric acid (PCA) extracts of rapidly dividing and growth-inhibited cells. HeLa cells were grown on tissue culture flasks at 37°C, 5% CO₂, in Dulbecco's modified Eagles medium (DMEM) with 5% calf serum. Mouse neuroblastoma cells N-115 (from Marc Kirschner) were grown on flasks at 37°C, 10% CO₂, in DMEM supplemented with glutamine (2 mmol per l of DME), and 10% fetal calf serum except as indicated. Whole cells were extracted twice with 0.74 M PCA: the extracts were combined and trichloroacetic acid was added to 20% to precipitate the proteins. The precipitate was washed twice with ethanol, dried and electrophoresed in polyacrylamide, acetic acid, urea gels according to Panyim and Chalkley¹⁶, except that the acrylamide was reduced to 7.5%. *a*, High density HeLa; *b*, rapidly growing HeLa; *c*, serum-deprived mouse neuroblastoma; *d*, rapidly growing mouse neuroblastoma.

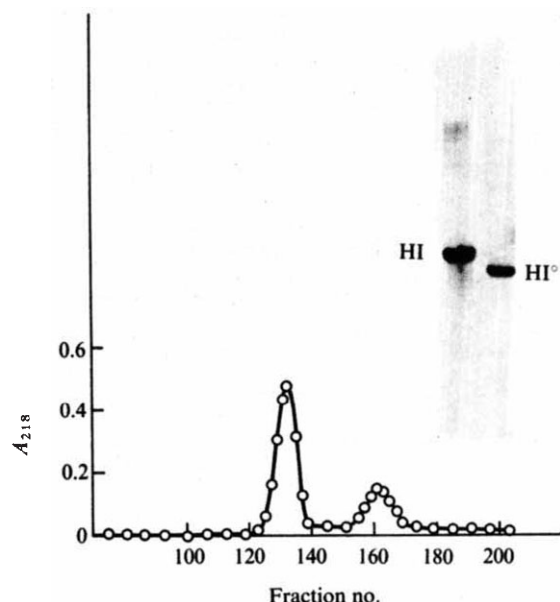


Fig. 2 Bio-Gel P-100 chromatography of PCA extracts. The 0.74 M PCA extract of serum-deprived mouse neuroblastoma cells was dissolved in 8 M urea, 0.01 M HCl and applied to a 2 × 150 cm Bio-Gel P-100 column equilibrated in 0.01 M HCl. The column was eluted with 0.01 M HCl at a flow rate of 6 ml h⁻¹. Fractions (1.2 ml) were collected, peaks were pooled, lyophilised and subjected to polyacrylamide electrophoresis, as shown inset. The left hand lane represents the earlier chromatographic peak, while the right hand lane corresponds to the other peak.

growing counterparts. A quantitative analysis of several such experiments is presented in Table 1. The increase of H1⁰ in both types of cells was about three- to fourfold when cell division was inhibited.

To prove the identity of the putative H1⁰, we purified it from PCA extracts of serum-deprived mouse neuroblastoma cells and submitted it to amino acid analysis. For this purpose we used chromatography on Bio-gel P100 (ref. 13), which was more rapid than the previously used Biorex 70 chromatography⁶. A representative chromatogram is shown in Fig. 2 along with electrophoretic analyses of the two peaks. The amino acid composition of the purified H1⁰ listed in Table 2 is like that of the H1⁰ reported by Panyim and Chalkley⁶.

Table 2 Amino acid composition of H1⁰

Amino acid	Mol %
Asp	3.9
Thr	5.4
Ser	9.1
Glu	6.1
Pro	8.2
Gly	4.3
Ala	16.6
Val	6.5
Met	0.7
Ile	2.4
Leu	2.1
Tyr	0.8
Phe	0.9
Lys	28.8
His	0.5
Arg	3.3

H1⁰ was hydrolysed in 6M HCl, 0.1% phenol for 20 h at 110°C. The figures are averages from two determinations using a Beckman amino acid analyser.

These results make it clear that histone H1⁰ content is not just a static characteristic of certain cells that become enriched in mitotically inactive tissues, but that H1⁰ levels are associated with a functional change in individual cells. Moreover, they suggest the possibility that H1⁰ is synthesised at times other than the S phase. In this regard it might be asked if H1⁰ ought to be compared to histone H5, whose synthesis is also not dependent on S phase¹⁴. The fact that both of these histones suppress chromatin activity adds interest to the comparison of H1⁰ and H5, metabolically, structurally and functionally. Such studies are under way.

We thank Gale Garcia for the culture of cells and June Holtz for amino acid analysis. This work was supported by NIH grants GMS 20338 and NIEHS 1 T32 ES07075.

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Continuous synthesis of long DNA chains by chick embryo DNA polymerase γ

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There is evidence for at least two mechanisms of DNA replication in eukaryotic cells. One is nuclear DNA replication in which DNA strands are synthesised in relatively short pieces (4–5S) that are later elongated and joined together^{1–6}. These short DNA intermediates are also observed in the replication of viral DNA such as polyoma virus^{7,8} and simian virus (SV40)^{11,12}, but not in adenovirus DNA^{13,19} and mitochondrial DNA¹⁵, where longer DNA chains are synthesised continuously. Studies of the subcellular localisation and the inhibitors of DNA polymerases^{16,17} suggest that DNA polymerase α , β and γ are involved in nuclear DNA replication, DNA repair and mitochondrial DNA replication, respectively. And it is suggested that DNA polymerase α and γ are involved in the replication of SV40 DNA¹⁸ and adenovirus DNA¹⁹, respectively. Does the difference between the two types of replication depend on the difference in the reaction property of DNA polymerases α and γ ? DNA polymerase α from human KB cells incorporated about 11 nucleotides per binding event²⁰. Using mouse enzymes we found that one DNA polymerase β molecule polymerises dTMP on the multiple oligo(dT) primers with poly(rA) templates in a highly discontinuous fashion, while DNA polymerase γ may synthesise rather long poly(dT) chains in a one enzyme–one product fashion. We report here that using nearly homogeneous chick embryo DNA polymerase γ , the results clearly indicating that one DNA polymerase γ molecule synthesises one long DNA in a highly progressive fashion.

DNA polymerase γ was purified from an extract of 11-day chick embryos by phosphocellulose column chromatography, ammonium sulphate fractionation, gel filtration on Sephadex G-200, hydroxylapatite column chromatography and native DNA–cellulose column chromatography. The final preparation has a specific activity of 570,000 units (nmol ³H-dTMP incorporated per h at 37°C) per mg protein with poly(rA) · oligo(dT) as a template · primer. A typical result of SDS-polyacrylamide gel electrophoresis is shown in Fig. 1. The major polypeptide which accounts for 86% of total protein is the polypeptide with a molecular weight (MW) of 47,000. This was the only polypeptide whose amount varied in proportion with the DNA polymerase activity during DNA–cellulose column chromatography, indicating that the 47,000-MW polypeptide was the only component of DNA polymerase γ . Purified DNA polymerase γ (149.4 U) was applied to SDS-polyacrylamide gel and the amount of the 47,000-MW polypeptide was determined as 0.224 μ g (Fig. 1). Therefore, the specific activity with respect to the 47,000-MW polypeptide is 660,000 U per mg protein. The result of gel filtration and the sedimentation coefficient (7.5S in the presence of 1 M KCl and 0.5% Triton X-100) indicate that the molecular weight of this enzyme is about 180,000 (data not shown), assuming that this enzyme is spherical (as our preliminary electron-microscopic observation suggests). Therefore, it is comprised of four identical 47,000-MW polypeptides. From the specific activity and the molecular weight of this enzyme, the turnover number is calculated as 1,980 nucleotides per min per enzyme molecule.

The size of poly(dT) products synthesised by the highly purified chick embryo DNA polymerase γ on poly(rA) · oligo(dT) was analysed by alkaline sucrose gradient centrifugation. Polymerisation of ³H-dTMP continued almost linearly for at least 2 h (Fig. 3a). The sizes of products synthesised in the reaction for 0.4, 0.9, 1.5, 2 and 3 min were 7.0S, 11.9S, 13.0S and 13.9S, respectively (Fig. 2). After 3 min, the rate of chain elongation levelled off and the size of products synthesised in a reaction lasting 10 min or longer was always 14.6S. Molecular weights of the products were calculated from sedimentation coefficients according to Abelson and Thomas²², and represented in nucleotide number (Fig. 3b). The initial chain elongation rate was about 1,900 nucleotides per min. The final length (average about 4,100 nucleotides) was reached at about 4 min—after that the size did not change but the number of products at the final length increased. Further studies are necessary to clarify how the final length is determined.

The coincidence of turnover number and DNA chain elongation rate indicates that one DNA polymerase γ molecule completes synthesis of one long poly(dT) chain before it starts with the other primer. The number of the products (equal to the number of dTMP molecules incorporated per average number of nucleotides per product) per enzyme molecule (number of enzyme molecules was calculated from the specific activity of the 'pure' enzyme, the molecular weight and the amount of enzyme

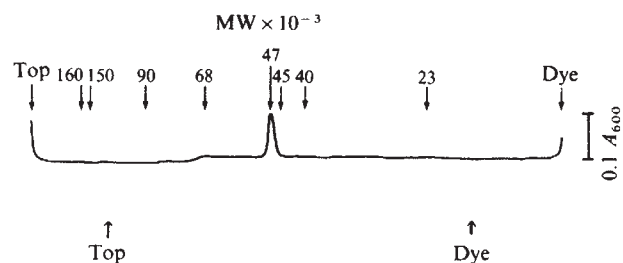


Fig. 1 SDS-polyacrylamide gel electrophoresis of the purified DNA polymerase γ . The detailed procedure for the purification of DNA polymerase γ from chick embryo will be published elsewhere. Extract was made by homogenisation, ultrasonication and centrifugation in the presence of 0.5 M KCl and 0.5% Triton X-100. Purification steps were: phosphocellulose column chromatography with stepwise elution; ammonium sulphate fractionation (0–55% saturation); a second phosphocellulose column chromatography with the elution in a linear salt gradient; gel filtration on a Sephadex G-200 column, hydroxylapatite column chromatography and native DNA–cellulose column chromatography. The preparation in the most purified fraction of DNA–cellulose column chromatography was used as the electrophoresis sample. DNA polymerase activity was determined as described in Fig. 2 legend DNA polymerase γ (149.4 units) eluted from a native-DNA cellulose column, which has a high specific activity of 570,000 U per mg protein, was precipitated with 15% trichloroacetic acid, redissolved in 20 μ l sample buffer containing 0.0625 M Tris-HCl (pH 6.8)/2% SDS/10% (v/v) glycerol/5% (v/v) 2-mercaptoethanol/0.001% bromphenol blue, then electrophoresed essentially as described by Laemmli²⁵, in which 10% acrylamide slab gel of 1 mm thickness with 5% condensation gel was used. After electrophoresis (8 h at 60 V), the gel was fixed and stained with 0.1% Coomassie brilliant blue in 10% acetic acid/25% isopropanol and was destained with 10% acetic acid/10% isopropanol. The gel was photographed (bottom) and scanned at 600 nm by a Fujiox scanning densitometer (top). The molecular weight of the polypeptide band was estimated by comparison of its mobility to those of marker proteins: *Escherichia coli* RNA polymerase containing subunits of β' (MW160,000), β (MW150,000), σ (MW90,000), and α (MW40,000); bovine serum albumin (MW68,000); ovalbumin (MW45,000); bovine α -chymotrypsinogen (MW23,000). The protein amount in the gel was determined by comparing with the intensity of stained standard proteins of the known variable amounts (0.1–2 μ g). Standard proteins were electrophoresed and stained in the same conditions as DNA polymerase γ . The average value of dye-intensity of three proteins; bovine serum albumin, ovalbumin and α -chymotrypsinogen, was used as the calibration standard.

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used) was one for the initial 3 min of the reaction, and then increased in proportion to reaction time (Fig. 3c and inset). One enzyme molecule synthesised 35 products in 120 min, indicating that the average time required to complete one product is 3.4 min, agreeing very well with the time of termination of chain elongation (Fig. 3b). Thus, the mechanism of DNA chain elongation of chick embryo DNA polymerase γ is highly progressive.

In contrast, a mouse DNA polymerase β molecule synthesises poly(dT) chains on many primers in a highly discontinuous way²¹. The number of products per DNA polymerase β molecule was always about 30 for the long reaction time, and the average size of all products increased slowly and consistently for at least 30 min²¹. The processive nature of DNA polymerase α is not so great as that of DNA polymerase γ (refs 20, 23). Furthermore, our unpublished data indicate that the DNA products synthesised by DNA polymerase α are short (about 3–4S), using mouse enzyme and single-stranded calf thymus DNA-3'-(dT)_n with oligo (rA) as a template-primer. Thus, the reaction properties of DNA polymerases α , β and γ are different from each other.

The behaviour of DNA polymerase γ in DNA chain elongation makes it suitable for the replication of mitochondrial DNA and adenovirus DNA which are synthesised continuously

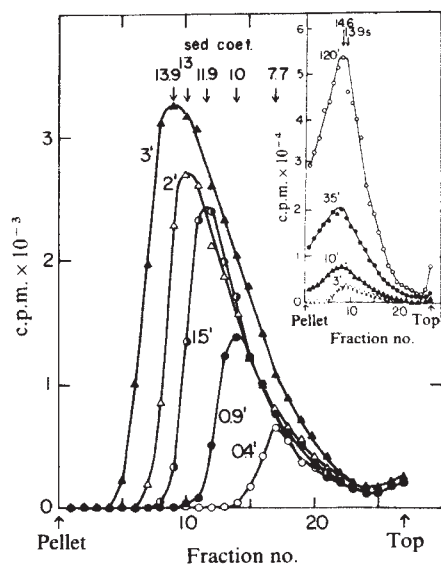


Fig. 2 Alkaline sucrose gradient centrifugation of poly(dT) products synthesised by DNA polymerase γ . The products were synthesised by highly purified DNA polymerase γ in optimal conditions. Reaction mixtures were incubated at 37 °C for 0.4, 0.9, 1.5, 2, 3, 10, 35 and 120 min in a final volume of 100 μ l contained the following components: 50 mM Tris-HCl (pH 8.5), 0.5 mM MnCl_2 , 1 mM dithiothreitol, 110 mM KCl, 20 mM potassium phosphate, 400 μ g per ml bovine serum albumin, 14% glycerol, 80 μ g per ml (rA)_n, 16 μ g per ml (dT)₁₂₋₁₈, 12.7 μ M ^3H -dTTP (1837 c.p.m. pmol⁻¹), and 0.65 U DNA polymerase γ . After incubation, a 5- μ l portion of each reaction was withdrawn to measure the amount of ^3H -dTTP incorporated into poly(dT) as described previously²¹. The other portion (95 μ l) was mixed with an equal volume of a 2 $\times$ denaturing solution containing 2 M NaCl, 0.6 M NaOH, 1.5% Sarcosyl and 30 mM EDTA, then incubated at 37 °C for 20 min. 100 μ l aliquots of the resulting solutions were layered over 4.8 ml 5–20% sucrose gradients made in the solution containing 1 M NaCl, 0.3 M NaOH and 5 mM EDTA, and then centrifugation was carried out for 15 h at 38,000 r.p.m. at 5 °C in an RPS 42T2 rotor of a Hitachi ultracentrifuge. The gradient was fractionated into 27 fractions from the bottom and a 100- μ l portion was placed on a DEAE-cellulose paper disk and washed as described elsewhere²¹. Radioactivity in poly(dT) retained to the disk was measured using a Beckman liquid scintillation counter. Authentic poly(dA) (8.5S) was centrifuged in an accompanying tube as a sedimentation marker, and the position was determined by measuring the absorbance at 260 nm.

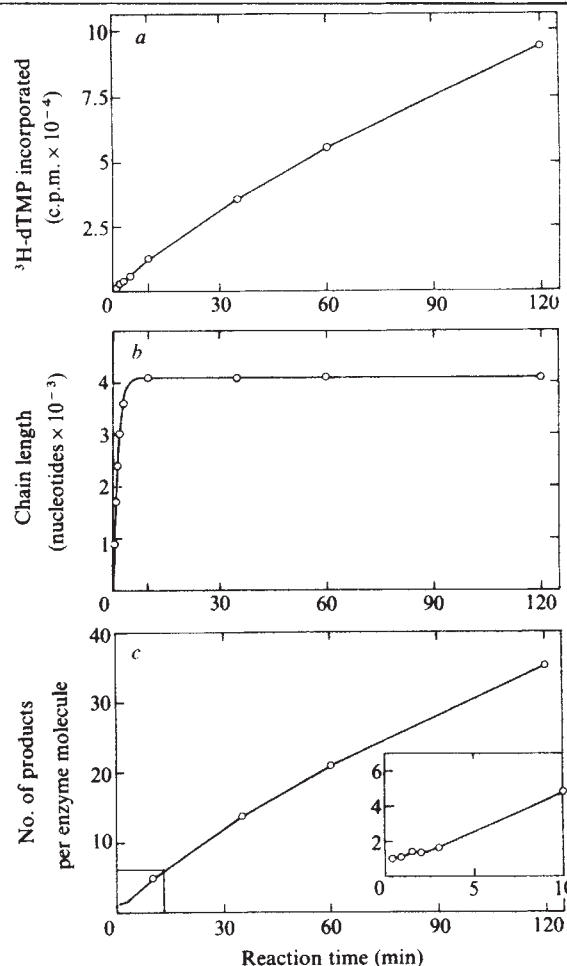


Fig. 3 The time course of incorporation of ^3H -dTTP into poly(dT) (a), elongation of poly(dT) chains (b) and the number of products per DNA polymerase γ molecule (c). Incorporation of ^3H -dTTP was measured as described in Fig. 2 legend. The chain length of poly(dT) products was calculated from the sedimentation coefficients as described previously²¹ and represented as the number of dTMP residues. The mole number of products was calculated by dividing the mole number of incorporated ^3H -dTTP by the chain length. The mole number of DNA polymerase γ was calculated from the unit of enzyme used, the molecular weight and the specific activity of the homogenous enzyme as described in the text.

without short intermediates. This is in good agreement with observations^{13,24} that have suggested that DNA polymerase γ is involved in the replication of mitochondrial and adenovirus DNA.

We thank Miss M. Nishizawa for assistance. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan and by a grant from the Research Foundation for Cancer and Cardiovascular Diseases, Osaka, Japan.

Received 12 December 1979; accepted 13 February 1980.

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Conservation of segmental variants of satellite DNA of *Mus musculus* in a related species: *Mus spretus*

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There has been much recent discussion on the biological function of the highly-repetitive DNAs (satellite DNAs) of higher organisms^{1–9}, that have several curious features of sequence organization. First, some satellite sequences show a high degree of conservation between related species^{10–15}. Second, there is independent evolution of 'type B' segments (sequence variants) within a block of tandem repeats¹⁶. The majority of the *Mus musculus* satellite DNA can be cut to produce a type A pattern with either *EcoRII* or *AvaII* (ref. 8). However, digestion of *M. musculus* satellite with other enzymes produces a limit series of fragments from only part of the total satellite (type B segment)¹⁶. To understand the significance of the segmental sequence variants, it is necessary to characterize their distribution between individual chromosomes of a genome¹⁸ and between closely related genomes. We report here the distribution of type A and B patterns in the two closely related species, *Mus musculus* and *Mus spretus*^{19,20}. The data show that the genome of *M. spretus* contains homologous sequences that are organized into the same type B segments as *M. musculus*, except for one type B segment that is under-represented in *M. spretus*. From a knowledge of the genetic distance and hybrid fertility between these species^{19,20}, we are able to exclude one of the proposed biological roles for these particular genomic components.

Analytical equilibrium sedimentation in neutral CsCl of total DNA of *M. spretus* that is bound to the dye Hoechst 33258 does not reveal a satellite DNA component (Fig. 1b) equivalent to that resolved from the total DNA of *M. musculus* sedimented in the same conditions (Fig. 1a). Hoechst 33258–CsCl density gradient centrifugation is a method that is particularly sensitive for the resolution of small amounts of AT-rich satellites^{17,21}.

To detect any 'musculus' type A or B patterns in *M. spretus* it is necessary to use a sample of pure total satellite DNA of *M. musculus*, that contains all sequence variants, in 'Southern' hybridizations²³ against *M. spretus* DNA after digestion with type A or B enzymes. In order to obtain a probe of high purity, total *musculus* DNA was resolved into satellite and main-band components after repeated fractionation in Hoechst 33258–CsCl gradients. The final purity of the satellite was monitored by restriction with a type A enzyme to produce the expected complete pattern of fragments (Fig. 2a) and by analytical centrifugation. On this basis we are confident that the *musculus*

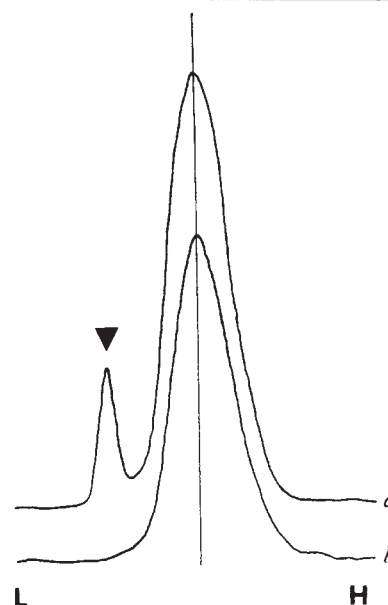


Fig. 1 Analytical UV absorbance profiles of Hoechst 33258–CsCl gradients after sedimentation to equilibrium for 18 h at 44,000 r.p.m. at 25 °C on a Centriscan 75 analytical centrifuge: a, *Mus musculus*; b, *Mus spretus*. 10 µg of DNA were bound to 10 µg Hoechst 33258 in the presence of 1% Sarkosyl²¹. CsCl was added to an initial density of 1.64 g cm⁻³. Arrow indicates *M. musculus* satellite band. H and L indicate heavy and light sides of the gradient respectively.

type A and type B variants detected by the probe in *M. spretus* are unlikely to be due to the presence of other contaminating components. A type B pattern of the probe is shown in Fig. 2b.

The majority of the *M. spretus* sequences that are homologous to the *M. musculus* satellite were susceptible to *AvaII* in that little homologous material remained at the origin (Fig. 3a). The amount of homologous 'musculus' sequences in *M. spretus* has been estimated as ~1% (at this stringency of hybridization) both

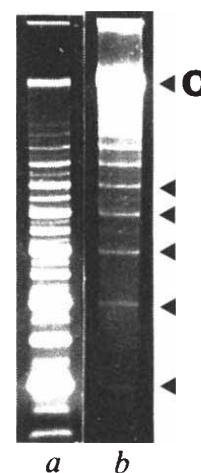


Fig. 2 Type A and type B patterns in *M. musculus*. Ethidium bromide stained agarose gel after *AvaII* digestion (a) and *HinfI* digestion (b) of *M. musculus* satellite DNA. Arrows indicate an ascending series of multimers based on a monomer length of approximately 240 base pairs (lowest arrow). O indicates undigested material left near the track origin. Purified satellite was prepared as described previously¹⁷ and 5 µg digested for 2 h with 2 units *AvaII* in 80 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, 30 mM NaCl at 37 °C, or, digested for 2 h with 2 units *HinfI* in 6 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 100 mM NaCl, 6 mM 2-mercaptoethanol at 37 °C. Digests were fractionated on a 1.6% agarose gel (15 × 15 × 0.3 cm) using a Tris-borate buffer (Tris 89 mM, boric acid 89 mM, Na₂EDTA 2.5 mM). Current loading was at 20 mA for 30 min and then increased to 40 mA (10 V cm⁻¹) for 3 h. Gels were stained with ethidium bromide and visualised on a short wave UV transilluminator and photographed.

from the exposure times needed to produce in *M. spretus* patterns of equivalent intensity to the *M. musculus* control and by the amount of radioactivity (detected by scintillation counting) on the *M. musculus* and *M. spretus* filters.

Southern hybridisations using *TaqI*, *AluI*, *Hinf*, *MboI* and *EcoRI* produced type B patterns similar to *M. musculus* in that only a portion of the homologous sequences were cut into bands and the rest remained as a dense band close to the origin (Fig. 3b-l). All enzymes, apart from *AluI*, cut a similar proportion of 'musculus' sequences in *M. spretus* to that cut in total *M. musculus* satellite: *TaqI*, ~35%; *Hinf*, ~10%; *MboI* and *EcoRI*, ~3% (as estimated from microdensitometer tracings). The results clearly indicate that the sites for *AluI* are under-represented in *M. spretus*: *Hinf* and *AluI* cut very similar proportions of *M. musculus* satellite but in *M. spretus* the bands produced from *AluI* cuts 2-3% of the *M. spretus* sequences and *EcoRI* 1-2% approximately. With the use of double enzyme digestions (Fig. 3c, e, h, k) we have investigated the overlap of some of the type B segments in *M. musculus* and *M. spretus*. The data provides no evidence for a substantial overlap of the type B segments in either of the two species. In all double digests no new prominent bands were observed. Overlapping type B segments would give rise to new bands due to the presence of different type B restriction sites lying at different positions within the basic 240-base pair repeat. Furthermore, the double digest bands are of greater intensity than the single digests,

indicating that the two respective enzymes cut largely non-overlapping portions of the total population of repeats.

Our failure to detect a satellite DNA in analytical Hoechst 33258—CsCl gradients of *M. spretus* (Fig. 1b) suggests that although similar distributions of restriction sites have been maintained in the two sets of homologous sequences, there has also been sequence modification that now renders the two sets differentially susceptible to Hoechst 33258.

Our results suggest that there are considerable differences in amounts of satellite DNA and some differences in organization and sequence composition between the genomes of *M. spretus* and *M. musculus*. It is possible that the presence of *musculus* type B patterns in *M. spretus* indicates the earlier evolutionary introduction of these patterns in a progenitor genome. The differences in amounts would be the result of independent evolution of the homologous sequences within each of the genomes subsequent to divergence. Given the broad similarity of the relative amounts of the type B segments in *M. spretus* to those in *M. musculus*, it may be that there has been a corresponding and equivalent reduction in satellite sequences at every centromere in *M. spretus*; or, alternatively, an amplification in *M. musculus*. However, the under-representation of *AluI* sites in *M. spretus* suggests that some chromosomal specificity may underlie the overall changes. Differences in abundance of the type B segments and some differences in the frequencies of type A multimers between an

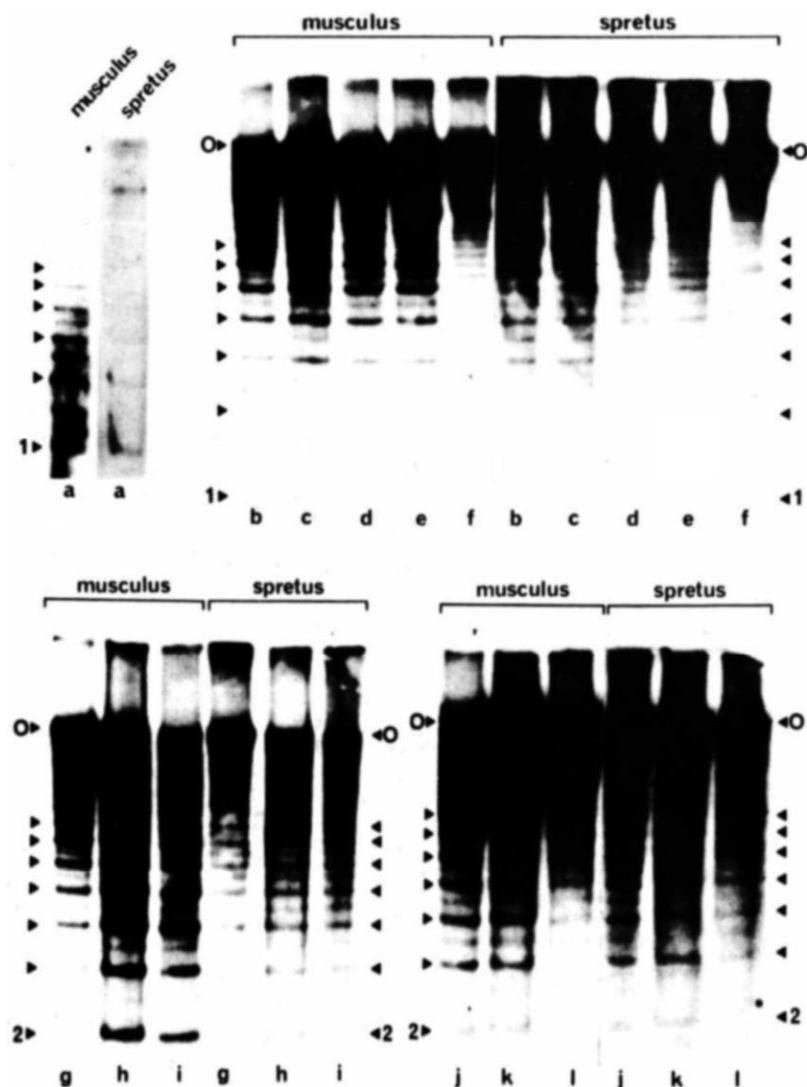


Fig. 3 Type A and type B patterns in *M. musculus* and *M. spretus*. Autoradiographs of digests of total DNA that have been fractionated on agarose gels, transferred to nitrocellulose and hybridised to ^{32}P -labelled *M. musculus* satellite. *M. spretus* lanes have been exposed 5-10 times longer than *M. musculus* to achieve a similar intensity. For clarity, different blocks of filters from the same species have been exposed for different times. Thus, for example, *M. spretus* g-i is a relatively shorter exposure than *M. spretus* j-l. All tracks have been exposed sufficiently to reveal the full arrays of bands of lower molecular weight. This has entailed a degree of overexposure of the high molecular weight material. Arrows indicate an ascending series of fragments based on multiples of a monomer length of ~240 base pairs. The multiple of the lowest arrow is indicated. Hybridization at the monomer position was barely detectable in all autoradiographs due to; first, the inefficient retention of fragments of this size during the hybridization reaction and, second, the small percentage of these fragments produced in a type B pattern (see Fig. 2b). Where there has been barely detectable hybridization at the monomer position this region of the autoradiograph has been omitted from some of the lanes (g-l). O indicates undigested material left near origin. a, *AvaII*; b, *Hinf*; c, *Hinf* + *AluI*; d, *AluI*; e, *AluI* + *EcoRI*; f, *EcoRI*; g, *Hinf* + *TaqI*; i, *TaqI*; j, *Hinf*; k, *Hinf* + *MboI*; l, *MboI*. Purified *musculus* satellite was nick-translated²² with [α - ^{32}P]-labelled dATP and dCTP (350 Ci mmol⁻¹; Amersham) to a specific activity of 10^8 c.p.m. μg^{-1} . Digests of total DNA (6 μg) were run on 1.6% gels with a Tris-borate buffer (see above); conditions of digestion were *AvaII* (as above); *Hinf* (as above); *MboI*, 6 mM Tris-HCl (pH 7.9) 6 mM MgCl₂, 6 mM 2-mercaptoethanol; *TaqI*, 6 mM Tris-HCl (pH 7.4), 6 mM MgCl₂, 1 mM dithiothreitol at 50 °C; *AluI*, 6 mM Tris-HCl (pH 7.7), 6 mM MgCl₂, 50 mM NaCl, 6 mM 2-mercaptoethanol, 100 $\mu\text{g ml}^{-1}$ bovine serum albumin at 37 °C; *EcoRI*, 100 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NaCl at 37 °C. Digestion was with 5 units of enzyme, for 2 h. Gels were denatured and neutralized according to Southern²³ and transferred overnight at room temperature to nitrocellulose paper (Schleicher and Schull, BA 85). Filters were baked *in vacuo* at 80 °C for 2 h. Whole filters representing 10 gel slots were hybridized with 12 ml of hybridization mixture containing 2×10^7 c.p.m. of ^{32}P -labelled *M. musculus* satellite DNA after it had been denatured at 100 °C for 10 min. Conditions of hybridization were 5 $\times$ SSC, 50% formamide, 0.5% SDS at 37 °C overnight with gentle agitation. Filters were washed extensively in 3 mM Tris base (unneutralized), dried and autoradiographed using preflashed Fuji X-ray film backed by Fuji MachII intensifying screen.

isolated X-chromosome cell line and the total genome of *M. musculus* suggests that independent evolution of sequences is occurring, not only within each species genome, but also between chromosomes of a species¹⁸.

The surprising finding, however, is that all recent sequence amplifications in either single chromosomes or in whole genomes address themselves to patterns that are already present within the genome and do not randomly generate new ones. In similar manner, recent data on the detailed organization of sequences that are shared between groups of species of Old World primates¹⁵ and between chromosomes and species of Gramineae²⁴ show that there are related sets of conserved sequences, with more recent amplifications of particular sequence patterns occurring within each species or species group.

Received 3 August 1979, accepted 5 March 1980.

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It is too early to specify the reasons for such evolutionary conservation and periodic amplifications (although speculation abounds), in our ignorance of the biological consequences of variation in types, amounts and distribution of sequence patterns. However, it seems unlikely that the differences in amount of satellite DNA in *Mus* are directly involved with the determination of chromosome homology during meiosis. These two species hybridize in the laboratory to produce fully fertile females and the species status of these two sympatric species is probably due to the evolution of premating isolation mechanisms¹⁹.

We thank Dr Bonhomme for providing *M. spretus* livers and introducing us to his studies. S.D.M.B. acknowledges the receipt of a NI SRC postgraduate studentship.

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Raman difference spectroscopy of tertiary and quaternary structure changes in methaemoglobins

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There have been several resonance Raman scattering investigations of the effect of inositol hexaphosphate (IHP) on methaemoglobins^{1–5}. In those studies the sensitivity for detecting frequency differences was limited to 1–2 cm⁻¹, and consequently frequency differences were not detected although spectral intensity differences due to changes in spin equilibria were. Shelnutt *et al.* recently reported on the observation of frequency differences induced by changes in the quaternary structure of chemically modified deoxyhaemoglobins⁶. An improved Raman difference spectroscopic technique⁷ with 0.1 cm⁻¹ sensitivity allowed the detection of these differences. We report here the application of this technique to a series of methaemoglobins with and without the addition of IHP. In addition to the intensity changes resulting from changes in the spin equilibria, we have observed frequency differences. In all liganded methaemo-globins that we examined a decrease in frequency of the mode in the 1,370 cm⁻¹ region was observed on addition of IHP. In those in which a quaternary structure change is known to occur the frequency difference is greater than 0.5 cm⁻¹. In those in which no quaternary structure change occurs [metHbA(CN⁻) and methHbA(N₃⁻)] the frequency difference is smaller (~0.15 cm⁻¹).

Human adult haemoglobin (HbA) was isolated and purified by standard procedures⁸. The ferric form was made by oxidation with NO₂⁻ followed by extensive dialysis or column chromato-

graphy to isolate the purified methaemoglobin. This stock solution was stored at 4 °C until ready for use when it was diluted in 0.1 M bis-Tris buffer to yield a haem concentration of between 50 and 100 μM. When IHP was added its concentration was between 4 and 10 times the haem concentration. The ligands were added so as to attain 0.1 M levels. Resonance Raman data were obtained on previously described instrumentation designed to have a high sensitivity for detection of small differences by simultaneous data gathering from two samples and subsequent analysis on a minicomputer^{6,7}. Data were obtained with 4,131 Å excitation from a krypton ion laser.

The Raman line of the porphyrin macrocycle in the 1,350–1,380 cm⁻¹ region has been well characterised as an indicator of oxidation state⁹, and, for low-spin ferrous porphyrins, to display a correlation with the degree of back-donation from the iron atom¹⁰. Shelnutt *et al.* recently reported that in deoxyhaemoglobins this Raman line is an indicator of quaternary structure⁶. They also found that in liganded proteins the line can serve as a measure of conformational stabilisation⁶. Because of these results the frequency of the line also might be expected to be sensitive to the quaternary structure change in methaemoglobins. We have examined several liganded methaemoglobins and found this to be the case.

In Fig. 1 the behaviour of the ~1,370 cm⁻¹ line is displayed for several methaemoglobins on addition of IHP. MetHbA(H₂O) and metHbA(F⁻), both of which have a well characterized quaternary structure change on addition of IHP^{11,12}, show clear frequency differences of 0.73 and 0.63 cm⁻¹ respectively (Table 1). The additional broad line in metHbA(H₂O) at ~1,355 cm⁻¹ in the difference spectrum results from deoxyhaemoglobin preferentially formed in the presence of IHP by photoreduction¹³. In both metHbA(H₂O) and metHbA(F⁻) the addition of IHP does not result in a linewidth change but instead the entire line shifts to lower frequency. The metHbA(H₂O) data are displayed to demonstrate this fact by superposition of the two spectra thereby allowing visual observation that both lines have the same width and that the Raman line in the sample containing IHP has the lower frequency.

For some of the other liganded methaemoglobins, complex line-shape changes occur on addition of IHP. For metHbA(Im⁻) there is a broadening of the line at 1,374.4 cm⁻¹ when IHP is present. From optical, circular dichroism, and additional Raman

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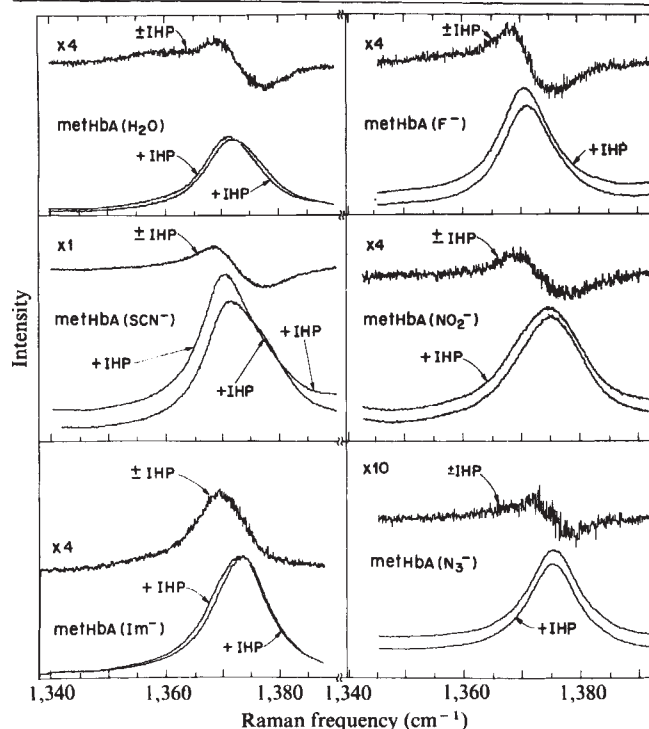


Fig. 1 Raman spectra and Raman difference spectra of the line in the 1,370–1,380 cm^{-1} region for a series of methaemoglobins with and without IHP. The line at 1,355 cm^{-1} present in the difference spectrum of methHbA(H_2O) results from photo-reduction in the presence of IHP. The methHbA(Im^-) difference spectrum was balanced so as to cancel the unchanged components of the lines.

measurements it has recently been found¹⁴ that this broadening results from a change in equilibrium in which additional methHbA(H_2O) is formed when IHP is added. This conclusion is supported by the difference spectrum in Fig. 1 in which a line appears at about the methHbA(H_2O) frequency. Because of this change in equilibrium we are unable to determine if there is a small change in frequency of the low-spin methHbA(Im^-) component. MethHbA(SCN^-) (see Fig. 1) and methHbA(OCN^-) are both of mixed spin character¹⁵ and have very asymmetrical lines in the 1,370 cm^{-1} region consisting of major peaks at 1,371.7 and 1,370.7 cm^{-1} respectively, and high frequency shoulders near 1,377 cm^{-1} . As seen from Fig. 1 in methHbA(SCN^-) a substantial change in shape and shift in frequency (-1.5 cm^{-1}) occurs on addition of IHP. The effect of IHP on methHbA(OCN^-) could not be determined because carbamylation at the N-terminus inhibits IHP binding. The influence on these data of changes in spin equilibrium, changes in the relative ligand/ H_2O binding equilibrium, and changes in binding to either the N-end or the S/O-end remains to be determined. MethHbA(NO_2^-) which is also mixed spin¹⁵ and in which it is not known whether binding occurs to one of the O atoms or to the N atom gives an increased broadening on addition of IHP and a change in frequency of -0.5 cm^{-1} . On addition of IHP to methHbA(N_3^-) (see Fig. 1) and methHbA(CN^-) the frequency decreased by 0.15 and 0.18 cm^{-1} respectively.

In a preliminary experiment on the ferrous protein, HbA(NO), we found that the addition of IHP also resulted in a lowered frequency ($\sim 1 \text{ cm}^{-1}$) of the 1,375.8 cm^{-1} line and a broadening as well. A complex change of shape in this case is expected because the coordination of the iron is known to change on addition of IHP^{16,17}. In a recent study by J. M. Friedman and K. B. Lyons¹⁸ the T structure of HbA(CO) was formed by partial photolysis of HbA(CO) in conditions of low CO partial pressure. In these experiments¹⁸ a 1–2 cm^{-1} shift to lower frequency of the 1,373 cm^{-1} line was detected.

To determine if frequency differences occurred in other Raman modes, we have examined the higher frequency region

of the spectrum of methHbA(F^-) and methHbA(H_2O) (Fig. 2). MethHbA(F^-) has a fully high spin ground state and methHbA(H_2O) has mixed spin character with a sufficiently small energy separation between the low and high spin states that the two states are populated according to their spin degeneracies at room temperature¹⁹. From magnetic susceptibility measurements determined by NMR¹⁵ addition of IHP does not alter the spin distribution of either of these liganded species. With F^- as the ligand, when IHP is added there is a decrease in intensity of the line at 1,478 cm^{-1} , an increase in intensity at $\sim 1,490 \text{ cm}^{-1}$ and a shift to higher frequency of the line at 1,564 cm^{-1} . Note, when considering the relative intensities of the 1,478 cm^{-1} line in methHbA(F^-) and methHbA(H_2O), the IHP-induced decrease in intensity of this line in methHbA(F^-) is consistent with a partial dissociation of the fluoride ligand and the consequent formation of methHbA(H_2O). Low frequency data (not shown here) on the line assigned⁴ as the Fe– F^- stretching mode also suggests such an interpretation. The behaviour of methHbA(H_2O) on addition of IHP is very different. For this case the intensities of the lines at 1,480 and 1,563 cm^{-1} increase on addition of IHP while the intensities of the lines at 1,505, 1,582 and 1,639 cm^{-1} decrease. The former frequencies correspond to the 'high spin' lines seen for example in methHbA(F^-) and the latter correspond to the 'low spin' lines seen for example in methHbA(CN^-). In spite of the qualitative difference in behaviour of these high frequency lines for methHbA(H_2O) and methHbA(F^-), the line in the 1,370 cm^{-1} region shifts to lower frequency in both cases.

The interpretation of the IHP-induced differences in the high frequency region of methHbA(H_2O) and methHbA(F^-) is intri-

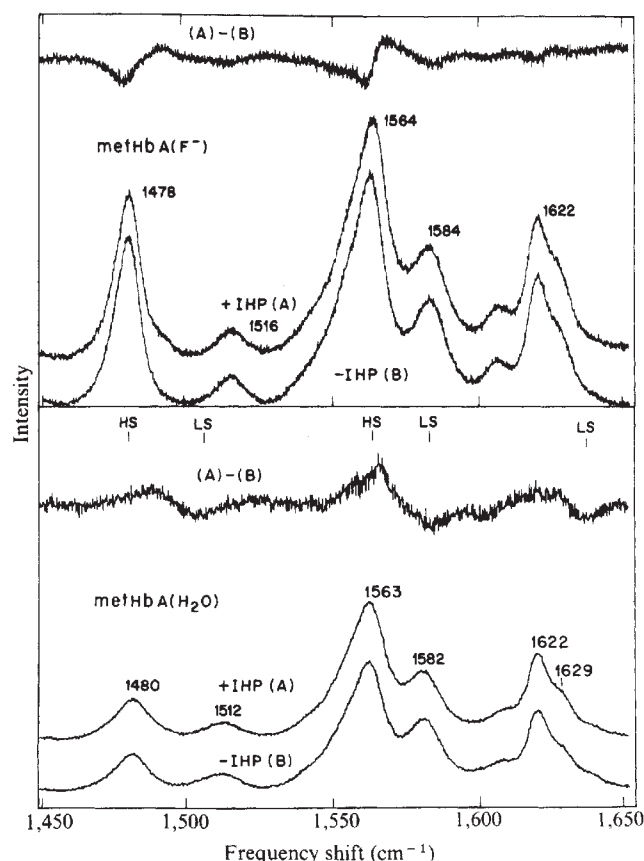


Fig. 2 Raman spectra and Raman difference spectra of the high frequency (1,450–1,650 cm^{-1}) regions of methHbA(F^-) and methHbA(H_2O) with and without IHP. The frequencies labelled HS and LS are those of high spin and low spin marker lines respectively. The scale of the difference spectrum for methHbA(F^-) is the same as that of the Raman spectra and for methHbA(H_2O) it is four times that of the corresponding Raman spectra.

Table 1 Properties of the structure dependent Raman line for several liganded haemoglobins

Ligand	Frequency	Width	IHP-induced difference
OCN ⁻	1,370.7	14.5	—
F ⁻	1,371.4	11.4	-0.63
SCN ⁻	1,371.7	14.5	-1.5
H ₂ O	1,372.0	12.5	-0.73
Im ⁻	1,374.4	11.5	—
CN ⁻	1,374.5	9.9	-0.18
NO ₂ ⁻	1,375.4	13.7	-0.54
N ₃ ⁻	1,376.1	10.6	-0.15
NO(Fe ²⁺)	1,375.8	13.1	-1.0

The frequencies were determined from the peak positions of the lines in the absence of IHP. The widths are the full width at half height. The IHP induced differences were all determined from the intensities in the difference spectrum. No difference is reported for OCN⁻ because carbamylation of the N-terminal residue blocks IHP binding. No difference is reported for imidazole (Im⁻) because IHP modifies the relative Im⁻/H₂O to haemoglobin binding constants.

guing. The crystallographically observed structural changes at the iron in metHbA(F⁻)²⁰ could account for the differences observed for this species. However an origin due to changes in the non-bonded interactions between the porphyrin macrocycle and the protein cannot be excluded. Observed spectral changes in deoxyhaemoglobins have been interpreted as originating from such an effect⁶. From NMR magnetic susceptibility measurements on metHbA(H₂O), it was found¹⁵ that the susceptibility is unaffected by the addition of IHP. On the other hand, from the Raman data reported here by using the 1,372 cm⁻¹ line as a reference we calculate a 10% increase in the intensities of the high spin marker lines when IHP is added to metHbA(H₂O). Such behaviour could result from changes in iron d-level energies, but these changes might be expected to modify the spin equilibria as well, and this would be reflected in the susceptibility measurements. It seems more likely that these changes result from a direct interaction between the protein and the porphyrin macrocycle. Such an origin for the changes we observe in metHbA(H₂O) also might account for the related observations in the visible absorption spectrum in which the transition in the quaternary structure from R to T produces an increase in the 'high spin' bands and a decrease in the 'low spin' bands¹⁵. Solvent-induced changes in the absorption spectrum originally thought to be signatures of spin state differences also have been observed in model haem compounds in the absence of spin state changes²¹.

It was previously observed in deoxyhaemoglobins that the Raman line in the 1,370 cm⁻¹ region was sensitive to changes in protein conformation⁶. The experiments reported here demonstrate that in methaemoglobins as well this Raman line is sensitive to tertiary and quaternary structural changes. In metHbA(N₃⁻) and metHbA(CN⁻) in which quaternary structure changes do occur, we observed IHP-induced frequency differences of about 30% of the differences in metHbA(F⁻) and metHbA(H₂O) in which quaternary structure changes do occur. This is approximately the same ratio as is observed for the UV difference spectra of these materials¹¹. In those methaemoglobins in which quaternary structure changes may be brought about by the addition of organic phosphate we observe differences of greater than 0.5 cm⁻¹. The Raman difference spectra may therefore be used as a quantitative indicator of quaternary structure change.

E.R.H. is a resident visitor at Bell Laboratories and was supported by the NSF (DMR-78-05916). S.R.S. was supported by grants from the NIH (HL-13527), the American Heart Association (76-688), and the New York State Health Research Council.

Received 27 December 1979; accepted 13 February 1980.

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Inhibition of photosynthesis by ethylene

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Little is known about how the plant hormone ethylene influences plant growth, except that it probably affects a fundamental control system. Research with inhibitors of ethylene binding and action has significantly expanded the level of understanding of the hormone. While CO₂, an inhibitor of ethylene binding, has been studied extensively^{1,2} the effect of ethylene on CO₂ metabolism in photosynthesis has been virtually ignored. An initial communication indicated no photosynthetic response to ethylene by either *Pisum sativum* L. or *Zea mays* L.³ but the exposure and the number of species tested were extremely limited. We therefore made a more extensive examination of the relationship between photosynthesis and ethylene. We report here a pronounced and reversible effect of hormonally significant levels of ethylene on the photosynthesis of the peanut.

Net photosynthesis, dark respiration and CO₂ compensation were measured on fully expanded leaves of 3-week-old plants of *Arachis hypogaea* L. cultivar Florunner. We used a semiclosed compensating system in which the amount of carbon dioxide supplied to maintain CO₂ at a given concentration was measured. Air circulated constantly through the plant chamber at 25 m min⁻¹. Chamber air temperature (25 °C) and vapour pressure deficit were controlled by the dewpoint principle, whereby humidified circulating air was brought to the desired dewpoint temperature and then reheated before entering the plant chamber. Light was provided from VHO cool-white fluorescent lamps supplemented with incandescent lamps giving 340 μEm⁻² s⁻¹ photosynthetically active radiation (PAR). The CO₂ content of the chamber was monitored by a Model 215 A-S Beckman infrared CO₂ analyser and held at a constant 300 ± 2 μl CO₂ per l of air by the compensating system. Carbon dioxide compensation was measured with the same system, however, without adjusting the CO₂ in the chamber.

The effects of light intensity were examined at both 1.5% and 21% O₂. We combined three sources of light: (1) the standard fluorescent-incandescent light banks used for routine plant growth; (2) 625 and 1,500 W quartz-iodine lamps; (3) a carbon arc, burning high-intensity photo 88 rods, which simulate the spectral composition of sunlight. The lowest light intensity was obtained by placing plastic shade cloth between the light bank and the plant chamber. This provided light levels of 180, 340,

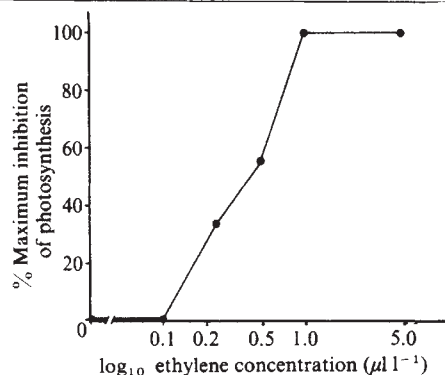


Fig. 1 Effect of a 2-h exposure to various levels of ethylene on the percentage maximum inhibition of photosynthesis of peanut leaves (25 °C, 300 μl CO₂ per l of air).

570, 920, 1,150 and 1,400 μEm⁻² s⁻¹ PAR, all below saturation of photosynthesis for this cultivar⁶. At any light intensity other than 340 μEm⁻² s⁻¹, the radiant energy was applied for 15 min. Light was increased in five steps from lowest to highest. (For a more detailed description of the methods and apparatus (see ref. 4).

A measured amount of ethylene (research purity, 99.9%) was introduced into the system using a hypodermic syringe with the internal level measured chromatographically (Perkin Elmer 881, FID) every 10 min. Samples of 1 ml of air from the chamber were separated at 110 °C on a column of activated alumina (1.83 m × 3.2 mm), with flow rates of 35, 30 and 550 cm³ min⁻¹ of nitrogen, hydrogen and air, respectively. During an experiment the ethylene metabolised by the plant and/or partitioned into aqueous phase was replaced.

Exposure of peanut leaves for 2 h to concentrations of ethylene (0.25, 0.50, 1.0 and 5.0 μl ethylene per l of air) resulted in a substantially reduced rate of photosynthesis (Fig. 1). The concentration of ethylene required to induce a decrease in photosynthesis was small and compared favourably with the concentration dependency kinetics of many ethylene-mediated growth responses⁵. A 2-h exposure of peanut leaves to 0.25 μl ethylene per l of air resulted in 33% of the maximum inhibition of photosynthesis, while 0.50 μl ethylene per l of air represented 56% of the maximum inhibition of photosynthesis.

The decrease in photosynthesis did not occur until 2.0–2.5 h after exposure and increased with length of exposure to ethylene (for example, 0.50 μl ethylene per l of air for 6 h resulted in a 39% inhibition in photosynthesis). The rate of photosynthesis returned to near normal with short periods of exposure (0.5–2 h) within 5 h of the initial exposure to the hormone.

Propylene, a structurally similar unsaturated hydrocarbon gas which can induce ethylene-like responses in some plants similar to that of ethylene¹, did not inhibit photosynthesis of the peanut

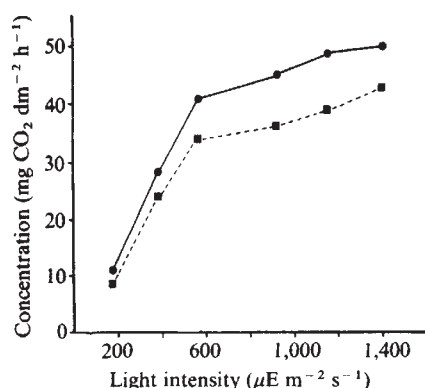


Fig. 2 Effect of light intensity on the inhibition of photosynthesis of peanut leaves by ethylene (1.0 μl l⁻¹) at 1.5% O₂ (25 °C, 300 μl CO₂ per l of air). ■, Ethylene; ●, control.

at a concentration (100 μl) propylene per l of air) 100 times that required to produce maximum inhibition by ethylene.

Photosynthesis was inhibited by ethylene (1.0 μl per l) at all light intensities tested (Fig. 2); however, the percentage of total inhibition of photosynthesis remained constant at each light intensity (~18%). These data (Fig. 2) represent photosynthetic rates at 1.5% O₂, showing that the decreased rate of photosynthesis cannot be accounted for by increased photorespiration. Similar curves, at lower rates of photosynthesis, were obtained at 21% O₂.

There was a significant change in the photosynthetic compensation point (Fig. 3). Exposure of peanut leaves to 1.0 μl ethylene per l of air increased the compensation point from 48 μl CO₂ per l of air to around 60 μl CO₂ per l of air. Because the stomata would be open at this level of CO₂, the inhibition of photosynthesis by ethylene did not seem to involve a stomatal controlled mechanism.

A decrease of photosynthesis in *Helianthus annuus* L. was also detected, whereas neither *Pisum sativum* L. nor *Phaseolus vulgaris* L. were effected by hormonal concentrations of ethylene. From past work⁵, the peanut would be expected to be the least susceptible of the three to such an effect. It has the least number of known responses to ethylene—inhibition of hypocotyl elongation and stimulation of hypocotyl diameter at 0.34 and 0.35 μl ethylene per l of air for 50% response saturation, respectively. The effect of ethylene on the photosynthetic rate of peanut and sunflower may be associated with their unusually high photosynthetic capacities^{6,7} and is being investigated.

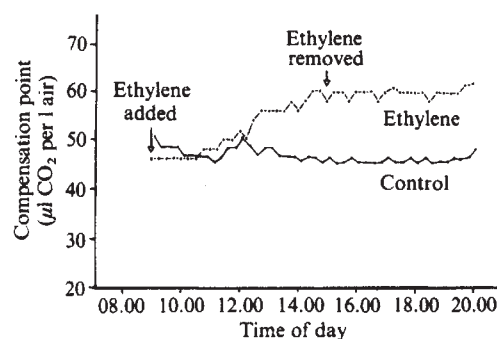


Fig. 3 Alteration of the photosynthetic compensation point of peanut leaves by 1.0 μl ethylene per l of air (25 °C).

An effect of ethylene on chlorophyll degradation has been reported at pharmacologically high concentrations⁸ (>10.0 μl per l of air). However, based on the relatively short exposure time for the induction of inhibition of photosynthesis by ethylene (2.0–2.5 h) and subsequent recovery, it is doubtful that an alteration in the rate of chlorophyll degradation and/or synthesis could account for the response we measured. This is supported by the work of Aharoni and Lieberman⁹ in which 3–4 d of exposure to 10.0 μl ethylene per l of air was required before a significant decrease in chlorophyll was detected in excised tobacco leaf disks. Alteration of chlorophyll degradation and/or synthesis, if it occurs in the peanut in these conditions, probably represents a secondary response which occurs substantially later than the initial molecular action of ethylene. In view of the diverse physiological responses of plants to ethylene, a more plausible explanation of the effect on photosynthesis would involve an effect on membrane permeability.

We thank D. Maxey and W. Turnbull for technical assistance.

Received 31 October 1979; accepted 6 February 1980.

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BOOK REVIEWS

The chilling story

H. M. Rosenberg

THE application of cold impinges on modern living in many ways and in this book David Wilson, the well-known radio and television science correspondent, describes the history and current developments of many aspects of refrigeration and cryogenic technology. The book is intended for the reader who has no specialized scientific background and the presentation is indeed clear and instructive.

After the introductory chapter which describes man's success in making fire as well as using cold, we are led into the seventeenth and eighteenth centuries — the world of Fahrenheit, Réaumur and Celsius. The measurement of temperatures and early methods of cooling below room temperature are described. This then leads on to a discussion on what is really meant by temperature and heat, and to the concept of an absolute zero of temperature. The third chapter is a fascinating account of the history of food preservation. The description of the development of the North American ice trade is particularly interesting. Ice was shipped not only from the northern to the southern states of the USA, but ice cargoes were even sent to India and Australia. With the increasing reliability of refrigeration machinery the ice trade declined, although natural ice was still harvested for food preservation until well into the present century. The saga of the development of refrigerated ships and the rise of the New Zealand meat industry is yet another part of the story that is extremely well told. The next stage was the mass production and distribution of frozen food to the general public. This started on a large scale with Thomas Wall's "Stop me and buy one" tricycles — 8500 of them — for selling ice cream to a mass market. But at that time few people had refrigerators and the gradual setting-up of the complete 'cold chain' from the freezing of fresh or prepared food to distribution via cold stores to the supermarket cabinet and then to the domestic freezer took another 40 to 50 years.

But nowadays we need temperatures even below that of frozen fish fingers, and the story of the liquefaction of the 'permanent gases' — nitrogen, oxygen and later on hydrogen and helium — is told with a keen sense of historical detail. It is sad to learn that Dewar, who was the first

Supercold: An Introduction to Low Temperature Technology. By D. Wilson. Pp.272. (Faber and Faber: London and Boston, 1979.) £8.50.

person to liquefy hydrogen, never really had an opportunity to score a double by liquefying helium, because of the row which developed between him and the man who had the only large supplies of helium gas in this country — Ramsay. And so credit for the first liquefaction of helium went to the great Dutch physicist, Kamerlingh Onnes of the University of Leiden, and on that date, 10 July 1908, the science of low temperature physics really began.

Although the extremely low temperatures now attainable with the liquefied 'permanent gases' catch the imagination, it is important to realize that the most widespread application of cryogenic engineering techniques is in the production and transport of gases. Gone are the heavy cylinders of compressed gas which are nearly as heavy when they are empty as when they are full. Nowadays oxygen and nitrogen are carried in bulk as liquids. Every large steel works has its liquefaction and fractionation plant in order to extract oxygen from the air — but the cold is wasted. Indeed, one of the problems of the gas producers is the surfeit of liquid nitrogen which is necessarily produced with the liquid oxygen. It is now being used as a refrigerant in the frozen food industry, in plants which shred old car tyres and as a cold bath for frozen bull semen. But by far the largest refrigeration enterprises are the plants which liquefy natural petroleum gas so that it may be stored and shipped from one country to another. The problems of liquefying and transporting such enormous quantities of potentially explosive liquid are extremely great and they have not yet been entirely overcome.

Another chapter is devoted to the problems of freezing biological material. Can we freeze living things solid, so that they revive when thawed out? There is no simple answer. Some fairly simple celled structures seem to survive freezing if they are first treated with glycerol, but whole animals, including humans, seem to be out. However it is possible to store animal embryos, thereby enabling us to ship a herd

of pedigree cattle to a far-off land in a Dewar flask of liquid nitrogen. But the storage of blood and of bull semen are the most successful products so far.

The final few chapters in the book deal with the properties of materials at liquid helium temperatures — within a few degrees of the absolute zero of temperature. From the technological angle the most exciting phenomenon is that of superconductivity — the complete vanishing of the electrical resistance in some metals and alloys. Superconductivity implies that we can pass electric currents through superconducting wires without any energy loss and this, in our conservationist world, is a very attractive idea. But although small prototypes of superconducting generators and motors have operated satisfactorily we are still some way from seeing a large superconducting generator in a power station. The prospect of a superconducting motor in a ship is intriguing — let us hope that sufficient funds will be made available to try it out! By far the most successful application of superconductivity up to now has been in the construction of extremely powerful electromagnets — magnets which produce fields which were almost unrealizable about 10 years ago are now available at a very reasonable cost. One exotic application of superconductivity which is not described and which should have been mentioned is the Japanese magnetically-levitated train using superconducting coils. A small prototype has been operated successfully. Computers with a very high packing of elements made from superconducting junctions seem to be promising but with these, as with any other application outside the scientific laboratory, the need for utter reliability is paramount, and it is far harder to make things foolproof at the temperature of liquid helium than at room temperature.

How low a temperature can we reach? To a certain extent it depends on what is meant by temperature. The lowest temperature to which a complete system in thermal equilibrium has been cooled is probably just below a thousandth of a degree, but a sub-system of nuclear spins can be magnetically aligned so that the effective temperature is only about a millionth of a degree above absolute zero.

Besides superconductivity other remarkable things happen at low

temperatures. There is the analogous effect in liquid helium which becomes superfluid at 2.17 K and for which we still do not have a completely satisfactory explanation. And, still colder, we have the remarkable series of changes which occurs in the light isotope of helium — liquid helium three.

This is a book which whets the appetite. The treatment is simple and yet the explanations are not so oversimplified that they would worry a scientist. Occasionally the broadcasting voice of the author comes

through a little too strongly so that the excitation and marvelling at new phenomena or techniques, which is very necessary via the microphone, is perhaps a little bit too enthusiastic in print. But this is only a minor point. David Wilson has done a good job and it is evident that he enjoyed doing it. □

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Molecular optical activity

S.F. Mason

The Molecular Basis of Optical Activity: Optical Rotatory Dispersion and Circular Dichroism. By E. Charney. Pp.376. (Wiley: New York and Chichester, UK, 1979.) £18.30, \$39.90.

FOR Louis Pasteur, the non-superposability of mirror-image molecular structures was the primary characteristic of natural products, as opposed to synthetic substances, and the distinctive physical property of handed molecules, their optical activity, provided him with a demarcation criterion between chemistry and life. The investigation of the optical rotatory power of chiral molecules, fundamentally, their differential interaction with left- and right-circularly polarized light, played a central role in the development of stereochemistry during the nineteenth and early twentieth centuries. From the 1950s, however, the chiroptical methods became increasingly overshadowed by more sophisticated physical techniques in structural chemistry, and their particular value in problems concerned with chiral recognition and discrimination was sometimes overlooked. The thalidomide tragedy, for example, could have been averted if this synthetic racemate had been separated into its optical isomers, for only the left-handed (S)-(–)-isomer has teratogenic properties. While occupying a subsidiary place in mainstream chemistry from the 1950s, the study of the electronic and structural basis of optical activity has proliferated at the periphery, in biophysics, biochemistry and chemical physics. The present book was written from the Laboratory of Chemical Physics, NIH, Bethesda, and the majority of the current theories of optical activity reviewed were first published in the *Journal of Chemical Physics*, rather than the orthodox physical chemistry journals.

The author's primary aim, which has been largely achieved, is the systematic development of the fundamental principles underlying the modern theory of the optical properties of chiral molecular

structures, and of the derived semi-empirical models employed in the stereochemical and other applications of chiroptical spectroscopy. After an introductory historical survey, the classical and the quantum theory of the interaction of an assembly of molecules with electromagnetic radiation is discussed, with the extension beyond the standard treatment required to account for optical activity, namely, the explicit consideration of terms dependent upon the ratio of the molecular dimensions to the wavelength of the radiation. The particular approximate models covered are those of the 'symmetric chromophore', or light-absorbing group, bonded to a dissymmetric array of substituent groups in a chiral molecule, the 'coupled chromophore pair' of a chiral dimer, and the general but more miscellaneous case of the 'inherently dissymmetric chromophore'. A group-theoretical treatment of chirality follows, with applications to the sector rules connecting the stereochemical configuration of an enantiomer with its rotary strength in a given electronic transition. There is a minor confusion in this section. The second of the three classes of point group symmetries listed (p.152) should be partitioned between the first and the third, since there are but two classes, chiral molecules with pure rotation symmetry and achiral structures with rotation-reflection symmetry, which implies the superposability of the mirror-image forms. Subsequent chapters are devoted to individual chromophore systems, the optical activity of polymers and of orientated molecule assemblies, and to recent developments. The latter include luminescence- and fluorescence-detected optical activity, and, more particularly, vibrational optical activity in both infrared absorption and Raman scattering spectroscopy.

The book is well illustrated, with a good selection of relevant absorption and circular dichroism spectra, and contains useful appendices of technical terms and of the unit systems in current use. It is recommended to all research workers concerned with chiroptical spectroscopy, as well as for general library reference. □

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Insight into disorder

Praveen Chaudhari

Models of Disorder. By J.M. Ziman. Pp.525. (Cambridge University Press: Cambridge, UK, and New York, 1979.) Hardback £25; paperback £12.50.

IN THIS book, Professor Ziman presents the first comprehensive and systematic review of the theoretical literature on the major forms of disorder. It is a remarkable book, both because of the subjects covered and because of the insight that the author brings to bear in explaining the generality and limitations of theoretical models.

The various forms of disorder are reviewed in the first three chapters. Chapter 1 is concerned with disorder where an underlying lattice can define the spatial arrangement of atoms. For example, compositional disorder in an alloy or spin disorder in an Ising model. This chapter contains a theme that is often repeated in the book — that of showing how the same mathematical model can be used to describe a number of situations which initially appear to be unrelated. In this instance the simple Ising model Hamiltonian is shown to apply to atomic interactions in a binary alloy. Its applicability to ferroelectric solids is briefly narrated. Chapter 2 deals with topological disorder characteristics of amorphous solids and liquids. Following a review of how the nature of disorder is described and modelled, there is a section on the analytical theories of the liquid state. In a natural progression, Chapter 3 contains a review of the statistical properties of random fields in continuous approximation. Chapter 4 has a brief and compact description of diffraction theory used in the study of disorder by experimental techniques. This is not a chapter that one can read and perform an experiment but rather one which contains the underlying physics and limitations on the information that can be extracted.

Chapter 5 has a detailed discussion of substitutional disorder. The success and limitations of the mean field approximations, the quasi-chemical approximation, the Bethe lattice and the pseudo-assembly method of Kikuchi are described. From these approximate solutions the author moves towards exact solutions of the one- and two-dimensional Ising model. The difficulties with obtaining a solution of the three-dimensional Ising model are touched upon and graphical methods are introduced. The chapter concludes with a succinct review of scaling and renormalization developments of the last few years. Chapter 6 deals with the thermodynamics of topological disorder. As in the preceding chapter, Professor Ziman begins with one-dimensional topological order and shows how genuine topological order cannot be

present in such models. He then proceeds to analyse the various approximations (for example, the van der Waal or the Percus-Yevick) used in coping with the difficult three-dimensional topological disorder. The role of computer simulation techniques in melting and the liquid state is emphasized, and the chapter concludes with a short discussion of the configurational and communal entropy and free volume.

Chapter 7 summarizes the statistical properties of polymers in solution. Branching, gel formation, rubber elasticity and random walks are some of the topics covered. The statistical treatments of the gels are connected to the Bethe lattice and the percolation problem. Chapters 8, 9 and 11 are concerned with excitations — magnons and phonons — in disordered solids. Chapter 8 develops the notion of localization or gaps in the spectral density using a linear chain model, and in Chapter 9 the discussion is extended to the three-dimensional case. Here the tight bonding and coherent potential approximation are discussed in some depth. Anderson localization and percolation theory and their significance in our understanding of disordered solids are presented. Chapter 11 contains a review of the dynamics of liquids and glasses.

Chapter 10 has a derivation of the resistivity equation (the Ziman formula) frequently used by experimentalists to explain the resistivity data on glasses and liquids. The generality and applicability of this deceptively simple formula is discussed. The remaining part of this chapter is concerned with scattering theories and their application to disordered solids. Chapter 12 contains a rather brief description of spin and ferromagnetic glasses. There is no mention made in this chapter of recent theoretical developments in spin glasses using topological notions. The final chapter contains the theory of metal-insulator transition and hopping conductivity.

Professor Ziman's book is primarily aimed at theoretical physicists. For readers with such a background or interest the book provides excellent reading of the current status of the field of disorder. The limitations of theoretical approaches, which are frequently assumed in the original sources, are clearly stated.

There are, however, a few areas which are either not adequately covered or are omitted entirely. Superconductivity and optical properties of disordered solids are not discussed. The theory of structural defects is barely mentioned. Considering the amount that has been covered these omissions are, perhaps, understandable. Overall, this book should be a very useful acquisition to anyone who is interested in the field of disorder. □

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Nuclear theory

Norman K. Glendenning

Nuclear Physics with Heavy Ions and Mesons. Edited by R. Balian, M. Rho and G. Ripka. Vol. I, pp.432; Vol. II, pp.548. (North-Holland: Amsterdam and New York, 1978.) Vol. I £66.75, Dfl.150; Vol. II \$80, Dfl.180; two volume set \$133.25, Dfl.300.

IN THE last half dozen years or so, the subject matter of nuclear theory has expanded remarkably, and no volumes that I know of chronicle this better than do these. The modern theoretical nuclear physicist becomes more and more a theoretical physicist as the techniques and concepts that he develops or borrows from other disciplines to understand nuclear behaviour under unusual conditions make intimate connections with other branches of physics. The articles are all excellent.

Volume I deals with collisions between heavy nuclei from low to relativistic energies. Semi-classical theory for peripheral low-energy collisions is

developed in several powerful approaches. The richness in behaviour of nuclear material under the stress of more intimate reactions or rapid rotation, or under the impact of relativistic collision, is the main subject.

For me, Vol. II is more exciting, perhaps only because the material is less familiar, but I think also because it indicates a new frontier of nuclear physics, where it blends with particle physics and with astrophysics. By now my copy is well worn. Most of this volume treats nuclear matter in the framework of a relativistic field theory for the light mesons and baryons, as is appropriate for studying new phases of matter at densities higher than normal such as exist in neutron stars or fleetingly at the site of a collision between nuclei at relativistic energy. This volume will be difficult going for the traditional nuclear physicist, but it opens the door to a new world.

The editors and authors are all to be congratulated for a fine work. □

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Three on food and land use

Joseph Hutchinson

The Growth of Hunger. By R. Dumont and N. Cohen. Pp.229. (Marion Boyars: London, 1980.) Paperback £3.50. *Green and Pleasant Land?: Social Change in Rural England.* By H. Newby. Pp.301. (Penguin: Harmondsworth, UK, 1980.) Paperback £2.50. *Farmland, Food and the Future.* Edited by M. Schnepf. Pp.214. (Soil Conservation Society of America: Ankeny, Iowa, 1980.) \$8.

I THOUGHT *The Growth of Hunger* was about hunger in the world getting worse. It is not. Repeatedly this is assumed, but nowhere is there any documentation of the extent of hunger, or any hard evidence given that it is more widespread now than it used to be. The book is, in fact, an attack on landlords, colonialism, the market economy, the Green Revolution, agrochemicals, multinational corporations, and poverty and injustice in society. There is already an extensive literature in this vein, and *The Growth of Hunger* does not add usefully to it.

In *Green and Pleasant Land?*, Howard Newby, after a decade of research into social change in rural East Anglia, writes perceptively, cogently, and with sympathy but not emotion. After an introductory chapter there are chapters on land and land ownership, the farming industry and the rise of agribusiness, and the farm worker

and the drift from the land.

There follow two chapters on the new influences that threaten the dominance of the agricultural interest in rural affairs. First is an account of the migration of urban-based families to rural domiciles, and second a discussion of the growth of environmentalism and its challenge to the privileged position of farming in planning legislation.

Newby probes deeply into the rural situation, and exposes the impotence and the growing isolation of the rural poor. The in-migration of urban people has generated a new affluent component in the rural population, and increased the polarization between rich and poor. With their cars and their town associations, the incomers have no difficulty in avoiding the isolation of the countryside, and they offer little support for rural services.

The incompatibility between the needs of the underprivileged and what it is economic to provide, is matched by the inconsistency between what the environmentalists regard as good and the agribusinessmen find is profitable. And having lucidly described these dilemmas, Newby leaves us. He has written a very good book, but now will he please write another. The conclusion I draw from *Green and Pleasant Land?* is that the social scientist — if he is as good as Newby — should advise us on what we should do, and the economist should then tell us what it would cost, and how we should pay for it.

Farmland, Food and the Future has nothing of the unity and coherence of *Green and Pleasant Land*. It is a series of essays sponsored by the Soil Conservation

Society of America on the loss of farm land to other uses. It was planned to draw attention to the facts of the loss of farm land, chiefly for urban uses, to consider whether they constituted a serious problem, and to report on what is being done about it. The essays are timely, since it is clear that the loss of farm land has only recently become a topic of concern in America, and there is not yet an agreed evaluation of the situation.

It is interesting and significant to compare the situation in the USA with that in England described by Newby. Many factors are similar. In both countries the

erosion of farm land to meet the demands of urban users is very great, and losses are heaviest from the best land categories. Both have experienced a long-continued drain of the rural population to the towns, and equally they now have to accept a reverse migration. Indeed, they are also alike in the propensity of the urban migrants to become defenders of the sanctity of the countryside. To quote: "Oregon is the victim of tremendous immigration, yet the newest immigrant is the first to want to slam the gate".

There are also fundamental differences. The underprivileged farm workers are the

historical consequence of the 'Master and Man' structure of English farming, which has no counterpart in the 'Family Farming' tradition of America. The American urban sprawl has been largely forestalled in England by planning legislation that would in America be an infringement of rights of property.

These essays will help to crystallize what is at present a rather amorphous debate.

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Flood adjustment

Malcolm D. Newson

Human Adjustment to the Flood Hazard. By K. Smith and G.A. Tobin. Pp.130. (Longman: Harlow, UK, 1979.) Paperback £3.95.

THE reviewer has just spent 48 hours monitoring the silt load of a Welsh river in high flood but, along with others who study the purely physical properties of flooded rivers, he is almost ashamed of the satisfaction such studies provide; to the general public and especially to those who suffer from disruption, damage or even bereavement from floods, they are nothing more nor less than a hazard to life. The authors' premise for this book is that the physical approach to floods, which has its eventual utility in incorporation by engineers into flood protection schemes, is divorced entirely in this country from the behavioural approach.

They begin their book by describing the flood as a hazard, adding financial figures in pounds or dollars lost by flood damage — as we all do these days to point to an economic justification for research! However, the striking thing is not that floods comprise 30% of all natural disasters, nor that they may be increasing in frequency, but that Man courts disaster so assiduously by developing floodplain sites for housing and industry. The book divides the remedies society provides into structural (mainly engineering solutions, but including land-use control upstream of the floodable area) and non-structural (insurance, flood-zoning and the fast-developing field of flood forecasting are included). By the middle of the book the largely review material is dispensed with in favour of a statement of the ideal form of comprehensive floodplain development, one in which hydrological technology is matched, not only with cost-benefit analysis, but also with studies of social feasibility and the whole operated on a planned basis. The original material, researched along the River Eden in Cumbria, describes how both Appleby

(a loss-bearing upland case) and Carlisle (a case of muddled floodplain adjustment) treat the threat of flooding from the Eden. The date of the most recent damaging flood is important in both local and regional studies (on the Eden it was 1968) and this makes the reviewer very anxious about how flood adjustment studies can ever become more widespread.

Other doubts about ever attaining the authors' ideals come from questionnaire survey results, illustrating just what an unmanageable lot we all are when confronted by hazard! Just as planner will never get all the people in one road to have grey front-doors they are unlikely to standardize the response to hazard. Here the authors' use of the word 'authoritarian' response to describe 'official' response to floods produces a

shudder that the cure might be worse than the ailment!

Admitting the bias of the reviewer, it is of great interest to read that advances in hydrology and in technology have now given Britain something of a lead in flood warning; after the Lynmouth disaster in 1952 no-one in the water industry believed we would ever be in a position to give an hour's warning of flooding; but now we could.

The book is well illustrated, copiously referenced and shows that, whilst the engineer and sociologist do not seem to be talking, the geographer holds a substantial key; will it be used to open doors outside Eden? □

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Protologic

Peter Bryant

The Origins of Logic (Six to Twelve Months). By Jonas Langer. Pp.437. (Academic: New York and London, 1979.) \$26.50, £15.

QUITE a common move in psychological research with babies these days is to look for what are called 'precursors'. The idea, most frequently applied to language acquisition, is that the things babies have to do and particularly the social routines which develop between them and their parents are sufficiently complex for them to pick up certain abstract rules which will help them a year or so later on when they begin to learn grammar. Analogies are made between the rules of the earlier social habits and grammatical rules, and it is argued that the one lays the basis for the other. This argument is often used in attacks on Chomsky's idea of an innate language acquisition device. The rules are learned not innately, it said, but they are learned before. The argument is attractive, but it has the flaw that it is very difficult to support empirically. How do you establish a causal link between the earlier and the later events? Simply to appeal to an

analogy between the two is glib.

The same unsolved problem, as well as several others, stalks the pages of Professor Langer's book, which is an attempt to discover the precursors of logic. He gives babies in their first year sets of objects to play with. If they put two things together here is said to be the precursor of addition — protoaddition it is called. Protosubtraction, protomultiplication, proto one-to-one correspondence, protoinference follow thick and fast. When a six-month-old baby puts a brick in his mouth, takes it out, but leaves his mouth open we are told that this is protosymbolic behaviour, because the gesture is detached from the object. The author hardly considers the possibility that what the babies are doing has nothing to do with logic, and he never questions his often-repeated assertion that the experiences they have playing with objects lays the basis for their later understanding of logic. Yet he gives no evidence, because no evidence is possible, for this central assumption. The observations are often acute, but the assumptions behind them, hedged though they are with impressive terms and often impenetrable prose, are very insecure. □

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OBITUARY

Roy Markham

THE DEATH of Professor Roy Markham, Director of the John Innes Institute, on 16 November 1979 has deprived us of a scientist who made outstanding contributions to research in several quite different experimental fields. He was born in London on 29 January 1916, the son of A.C.C. Markham, and educated at St Paul's School. In 1935 he entered Christ's College, Cambridge and read biochemistry for Part II of the tripos. After graduation in 1938 he decided on a research career, joining N.W. Pirie in the biochemistry department. In 1940 he joined Kenneth M. Smith at the Plant Virus Research Station which was then under the auspices of the Ministry of Agriculture.

By modern laboratory standards the available facilities at the Plant Virus Research Station were very primitive. They consisted of a few glasshouses and part of a wooden potato storage shed which had neither heating nor running water. However, they were fortunate enough to be allowed to continue making use of a room as a laboratory at the top of the Molteno Institute, previously allocated to Professor David Keilin. In this room, together with some additional facilities made available on the Downing site, Roy Markham laid the foundations for his later achievements. By 1942 he had already developed a steam distillation apparatus suitable for micro-Kjeldahl analysis which was subsequently listed under the British Standards Institute B.S.1428.

In 1940 Kenneth Smith was working with a new plant virus (turnip yellow mosaic) which was shown to have several interesting properties. Roy Markham became particularly interested in the physico-chemical properties of this and other small plant virus particles which stimulated his interest in the problem of high speed centrifugation. He had a genius for improvising and was highly skilled in making his own apparatus from bits and pieces found in the laboratory. An air driven high speed centrifuge was one of the devices he constructed to isolate virus particles.

It was soon realised that highly concentrated virus suspensions could be produced with a high speed centrifuge, and it was relatively easy to purify the virus with ethanol and half saturated ammonium sulphate. These highly concentrated suspensions led to the formation of octagonal crystals and this virus was one of the first (if not the first) insect-transmitted (flea beetle) viruses to be crystallised.

A number of important results emerged

from these early experiments; it was shown that turnip yellow mosaic virus could be separated into two fractions which were named "top" and "bottom" components. At the time there was considerable controversy concerning the presence of nucleic acid and its significance in virus particles, but Roy Markham clarified the issue by establishing that the "bottom" component containing the RNA was highly infectious whereas the "top" component devoid of the nucleic acid was non-infectious.

In 1945 the research came under the aegis of the Agricultural Research Council and the Plant Virus Research Unit was established. Roy Markham became increasingly involved with the physicochemical properties of plant viruses. Kenneth Smith concentrated more on the biology, ultrastructure and transmission of plant and insect viruses. However, Roy Markham, together with Ellis Cosslett were among the first investigators to record electron micrographs of plant virus microcrystals.

He will be particularly remembered for his work on viruses when he was joined by J.D. Smith and R.E.F. Matthews. They formed a most fruitful collaboration, producing a series of important publications which established the composition and properties of several plant viruses. He was elected Fellow of the Royal Society at the early age of forty.

His interest in electron microscopy started when examining air-dried and unstained samples of viruses in an early RCA machine which was installed in the Cavendish Laboratory. Although many biologists were highly critical of the images produced from early studies in electron microscopy, Roy Markham quickly realised the powerful potential of applying these instruments to the structure and measurement of virus particles. When the Plant Virus Research Unit was fully established at the University Farm in Huntingdon Road, Cambridge, the laboratory possessed one of the first production electron microscopes. In addition, extensive research facilities were made available with the aid of various grants Roy Markham had obtained in support of the expanding virus research programme.

Kenneth Smith retired in 1959 and Roy Markham became Director of the Plant Virus Research Unit. The work continued to expand and a regular flow of visiting scientists came to his laboratory. His interest and enthusiasm for developing and building apparatus gathered momentum as war surplus equipment continued to

provide an ideal source of materials. The symmetry associated with the architecture of virus particles together with their repeating features stimulated him to construct a device which allowed the regular structural features of viruses recorded in electron micrographs to be averaged and subsequently reinforced. The technique was established as the "Markham averaging method". In addition he investigated the potential of image analysis by building a simple ultra-violet light optical diffractometer for recording the diffraction spectra from electron micrographs and making accurate measurements.

There will be very many visitors to the Plant Virus Research Unit at Cambridge who will remember the warm welcome and hospitality they received from Roy and Margaret (née Mullen whom he married in 1940), who enjoyed entertaining their guests in a most elegant style. Roy Markham had a lighted-hearted approach to research, coupled with an infectious sense of humour which visitors from abroad found difficult to appreciate on first impression, and it must be said led to some misunderstanding on more than one occasion.

In 1967 the Directorship of the John Innes Institute fell vacant at a time when a decision had been made to move the institute from Bayfordbury in Hertfordshire to Norwich. Roy Markham was invited to fill the post, and saw an opportunity to create a unique centre for research by proposing that an institute could be established by moving the Plant Virus Research Unit in Cambridge to form part of the John Innes Institute at Norwich. This resulted in a considerable undertaking, as Roy Markham became involved in the planning of a large complex of laboratories and ancillary services. The John Innes Institute brought together under Roy Markham's Directorship and guidance during a period of just over ten years, has been re-established as a centre of international standing.

It is characteristic of Roy Markham that he left little record of his life, apart from his collection of scientific and administrative material. Although he had relatively few interests outside the laboratory, he devoted a great deal of his spare time at home to designing and constructing experimental apparatus for people within the institute. During his long illness he persisted until the end in working on a second and more advanced device for the processing of electron microscope images with the aid of computers.

R. W. Horne

T. L. McMeekin

THOMAS LEROY MCMEEKIN, commonly known as "Mac" to his friends, died in Columbia, South Carolina on 11 November 1979. He did notable work in protein chemistry over more than three decades, first in academic research and later as the head of an important government laboratory.

Born on 8 May 1900 in Monticello, South Carolina, he took his PhD at the University of Chicago in 1925, under the direction of Professor F. C. Koch, his thesis dealing with the purification of pepsin. In 1928, he joined the Department of Physical Chemistry at Harvard Medical School, headed by Professor Edwin J. Cohn. Cohn, whose primary concern was with the physical chemistry of proteins, was then engaged in purifying the substance in liver that was active in the treatment of pernicious anemia. This program achieved a clinically effective liver extract, though it failed to overcome the formidable problem of isolating what was much later to be known as vitamin B₁₂. McMeekin played a central part in this work, but his major contribution during his twelve years at Harvard came in studies on the physical chemistry of amino acids, peptides, and a wide variety of related molecules.

This cooperative enterprise involved Cohn, J. P. Greenstein, myself and other members of the laboratory, together with George Scatchard and John G. Kirkwood at MIT, and Jeffries Wyman at the Harvard Biological Laboratories. McMeekin's role was primarily in the choice and synthesis of a wide variety of organic compounds, chosen to define the relations between structure and relative solubility in water and organic solvents, and other properties such as partial molal volumes. The aim was to provide a basis for interpreting the unusual properties of amino acids and peptides, and (by implication) of proteins, in terms of their content and arrangement of polar, non-polar, and electrically charged groups. McMeekin's work was essential to all this; in particular his studies on the influence of non-polar groups on solubility in various media provided fundamental data and systematic relations for the understanding of what later came to be called hydrophobic interactions. In studies on proteins, he achieved the crystallization of two forms of albumin from horse serum.

In 1940 he joined the Eastern Regional Laboratory of the U.S. Department of Agriculture in Philadelphia, and later became director of its Protein Division. With R. C. Warner in 1942 he carried out a fundamental study on the hydration of β -lactoglobulin crystals, showing for the first time that it was possible to study protein crystals by direct analysis of water, salt, and protein, and to relate the composition of the crystal to that of the surrounding

medium. His studies on milk proteins — notably on β -lactoglobulins and on the separation and characterization of several components of casein — led to his receiving the Borden Award in 1950. Other important contributions from his laboratory involved the preparation of new types of protein fibers and study of partial specific volumes of proteins in relation to their structure.

On retiring from the Protein Division in 1965 he became Research Professor of Biology at the University of South Carolina in Columbia until 1972. He spent his last years near by, in Monticello, his birthplace, remaining active and alert until his final brief illness.

He was twice married, first to the late Vera Crockatt, and subsequently to Crystal Stribling, who survives him. He had two daughters and a son by his first marriage. One daughter, Dorothy McMeekin, is Professor in the Department of Natural Science in Michigan State University at East Lansing.

McMeekin was modest and gentle in manner; but his strength of character was obvious to those who knew him. He was ever helpful to his colleagues and to younger co-workers. He had an excellent quiet sense of humour, and a shrewd eye for the strengths and weaknesses of other human beings. Above all he was a man of complete integrity, who will be remembered with admiration and affection, especially by that band of protein chemists of which he was a distinguished member.

John T. Edsall

H. H. Plaskett

WITH THE DEATH of Professor H. H. Plaskett on 26th January 1980, Britain has lost one of its most distinguished astronomers.

Harry Hemley Plaskett was born in 1893, the elder son of Dr J. S. Plaskett, who later became Director of the Dominion Astrophysical Observatory in Victoria, B.C. After service in France during the First World War, Plaskett joined his father's observatory as a staff member, where he remained for eight years until he became Professor of Astrophysics at Harvard in 1928. He was appointed Savilian Professor of Astronomy in Oxford in 1932, but his productive years were again interrupted by war service between 1939 and 1944. Part of the later years of the war were spent by him in developing a new type of sextant for the Ministry of Aircraft Production, which was in constant use until quite recently.

Almost the whole of Plaskett's working life was spent in astronomical spectroscopy, beginning during his years in Canada with stellar spectroscopy and his pioneering work on the emission spectra of

gaseous nebulae. He had always recognised the importance of solar studies as a basis for understanding stellar atmospheres, and remarked in 1931 that "spectroscopic investigations of the sun seem likely to yield more information about stellar physics than spectroscopic studies of the stars themselves". In this year he published his classical observations of the variation of the profiles of the Mg b lines across the solar disk. This was an important step forward in astrophysics which involved the development and application of new and accurate techniques of spectrophotometry, many of them devised by Plaskett himself especially for this study. It also came at an appropriate time because of parallel work on the theory of line formation in stellar atmospheres being conducted by Eddington and Milne, following earlier work by Schuster. Plaskett's comparison of theory and observation led to a first good understanding of the mechanisms by which stellar spectrum lines are formed.

On his appointment at Oxford, Plaskett decided to devote himself wholly to solar studies. To this end he built an advanced solar telescope with a high dispersion spectrograph at the observatory. With it he planned a series of observations of photospheric mass motion through the Doppler displacements of special lines. Although this work was soon stopped by the Second World War, he felt so much encouraged by its progress after the war that he built a second solar telescope of aperture 20 in and focal length 35 m, complete with a fine spectrograph. With this he resumed his work on solar mass motions, the results of which, and their interpretation, were presented in a well known series of papers. During his 28 years in Oxford, Plaskett built up a small but distinguished group of solar astronomers, which was strengthened through close contacts with mathematicians and theoretical astronomers in other departments of the university. Over the years, many astronomers became indebted to him for the great help they received, and many owed the start of their careers to him.

Plaskett had a considerable influence on the development of astronomy in Britain. In a celebrated Presidential Address to the Royal Astronomical Society in 1946, he proposed the building of a large optical telescope in Britain. This resulted in the construction in 1968 of the 98 in Isaac Newton telescope at Herstmonceux, which is now soon to be moved to the new Northern Hemisphere Observatory in the Canary Islands.

After his retirement, Plaskett continued to work in the Department of Astrophysics and he worked regularly with the 20 in telescope until the age of 84 years. He was elected a Fellow of the Royal Society in 1936 and was awarded the Gold Medal of the Royal Astronomical Society in 1963. He is survived by his wife Edith, and his son and daughter.

D.E. Blackwell